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# Evaluation of the Aircraft Ground Equipment (AGE) at Pacific Air Force (PACAF) Locations

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- Kunsan Air Base, Gunsan – 8<sup>th</sup> Maintenance Squadron (MXS)
- Andersen Air Force Base, Guam – 36<sup>th</sup> MXS

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## List of Acronyms

AB	Air Base
AFB	Air Force Base
AGE	Aircraft Ground Equipment
BCO	Battelle Columbus, OH Operations
BLAST	Basic Local Alignment Search Tool
CDRL	Contractor Data Requirements List
CFU	Colony Forming Units
CONUS	Continental United States
COTS	Commercial-Off-The-Shelf
EDS	Energy-dispersive X-ray Spectroscopy
EMS	Equipment Maintenance Squadron
EPS	Extracellular Polymeric Substances
HALS	Hindered Amine Class)
IOB	Iron Oxidizing Bacteria
ITS	Internal Transcribed Spacer (gene)
LTPC	Low Temperature Powder Coating
MIC	Microbially Influenced Corrosion
MXG	Maintenance Group
MXS	Maintenance Squadron
NCBI	National Center for Biotechnology Information
PACAF	Pacific Air Force
PBS	Phosphate Buffered Saline
PDA	Potato Dextrose Agar
PTMLB	Portable Trailer Mounted Load Bank
PU	Polyurethane
PWS	Performance Work Statement
SRB	Sulfate Reducing Bacteria
TSA	Tryptic Soy Agar
USAF	United States Air Force
UV	Ultraviolet

## Executive Summary

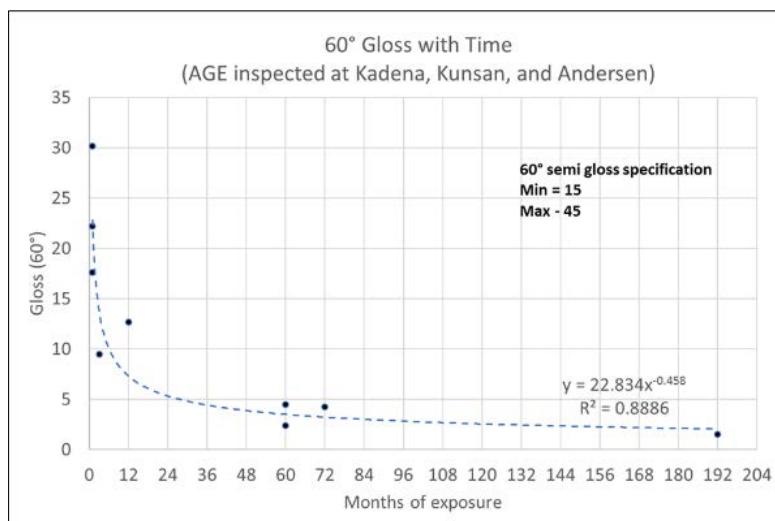
The United States Air Force (USAF) equipment and assets that are stationed in Pacific Air Force (PACAF) locations are exposed to highly corrosive environments resulting in damage of the equipment and adding costs for its upkeep. Battelle is working with Hentzen to modify their Commercial-Off-The-Shelf (COTS) Low Temperature Powder Coating (LTPC) 6191-61003. The objective is to tailor the COTS LTPC formulation to include suitable ultraviolet (UV) stabilizers to improve weathering performance and add relevant biocides to mitigate Microbially Influenced Corrosion (MIC).

This report is the deliverable for CDRL A002 of Phase II (PWS paragraph 4.3). The objectives were for Battelle to directly inspect, review and document aircraft ground equipment (AGE) conditions and environmental conditions at the following PACAF installations:

- Kadena Air Base (AB), Japan
- Kunsan AB, Korea
- Andersen Air Force Base (AFB), Guam

At each installation Battelle inspected five AGE assets for degradation of coatings from UV light and effects of corrosion in general and specifically, MIC. The selected AGE had coatings with varying levels of maturity ranging from 1 month to several years. Since MIC cannot be characterized by visual inspection, gauze samples were collected from corroded and non-corroded (i.e. background) areas of AGE using standard sample collection techniques, microbes were extracted, and were then characterized for potentially MIC causing bacteria and fungi species.

**Coatings Degradation from Exposure to UV light.** Color, gloss, and coatings thickness measurements were taken from the AGE inspected at the three PACAF locations. Results as seen in Figure ES- 1 show significant loss of gloss across the inspected AGE as quickly as 3 months after being painted. Gloss readings on the MIL-PRF-85285 coating were below the minimum gloss specification of 15 units for semi-gloss in the 60° angle of incidence. Readings for color showed significant change over time from UV exposure. The two parameters indicate high levels of UV degradation of the polyurethane backbone, which in turn reduces the binding between the PU and the pigments. This can cause chalking and roughening of the coating surface which reduces the gloss of the coating. The inspection of AGE at each PACAF locations and the respective readings to determine



**Figure ES- 1. Overview of UV Degradation**

level of coatings degradation from exposure to UV and, the general modes of corrosion are discussed in details in section 3.0 and evaluation of the PACAF AGE results are discussed in sections 4.1 - 4.3 of this report.

**Characterization of Corrosion Modes and MIC at PACAF Locations.** Several types of corrosion were observed in the inspection of AGE in the PACAF region and as stated earlier, all of the corrosion products

cannot be attributed to MIC. For example, most corrosion emanating from fasteners and metallic joints is likely crevice or galvanic corrosion or a combination of the two modes. Preferential weld corrosion was observed on stands and similar AGE where the metal used to make the weld is slightly anodic compared to the parent metal. Therefore, the weld metal corrodes at a higher rate than the parent. Corrosion appearing through paint at corners and edges is likely a result of edge effects and the coating naturally being thinner there, so it is the first spot to form pores and leads to corrosion propagation along the edge. Corrosion observed with paint bubbling up is likely filiform corrosion but could be MIC as well. Therefore, the analysis of the microbial samples was critical for determination and for characterizing the MIC. The microbial samples from corroded areas of the inspected AGE showed no major fluctuations in the bacterial species / activity among the three PACAF locations. Kadena AB had the largest sample to sample variability, while Andersen AFB had the least. Equivalent levels of bacterial species were characterized on several samples from the non-corroded areas of the AGE, although the microbial levels observed on the non-corroded AGE samples at Andersen AFB was found to be approximately 2 logs lower than those found from Kadena AB and Kunsan AB. This is expected as bacteria and fungi can be found essentially everywhere and in equivalent amounts. *Sphingomonas* were the most prevalent across all of the PACAF locations, followed by *Massilia*. *Pseudomonas/Brevundimonas* was quite prevalent across all PACAF locations and is also known to contribute to MIC, likely due to its ability to form biofilms. *Acidovorax/Variovorax*, a known iron oxidizing bacteria (IOB) as well as a biofilm producer and *Methylobacterium*, known to cause corrosion in copper pipes and therefore likely contributors to MIC were not nearly as prevalent as the other two on the PACAF samples. A summary of results is presented in Table ES- 1.

**Table ES- 1. Potential MIC-causing Bacteria Prevalent Across All PACAF Locations**

Prevalent Bacteria Across the Three PACAF Locations	Mechanism Influencing MIC	Cell Wall Type
<i>Sphingomonas</i>	Degradates alkane, Copper-degrading, Nitrate-reducing	Gram Negative
<i>Acidovorax / Variovorax</i>	Biofilm formation (Variovorax), Copper-degrading (Variovorax), Iron-oxidizing (Acidovorax), Nitrate-reducing (Acidovorax)	Gram Negative
<i>Pseudomonas / Brevundimonas</i> <sub>9</sub>	Forms biofilms, Stainless steel corrosion, Copper-degrading, Nitrate-reducing, Carbon Steel degrading	Gram Negative
<i>Massilia</i>	Found in MIC-causing consortia, Copper-degrading	Gram Negative
<i>Methylobacterium</i>	Copper corrosion	Gram Negative

The *Exophiala* genus was by far the most abundant fungal species across the three PACAF locations. The results for other species were more scattered across the locations. *Dothideomycetes* were found on the AGE samples from Kadena, but were not quite as prevalent on the AGE in Kunsan and Andersen. *Toxicocladosporium/Cladosporium* and *Aureobasidium* were also found across all the PACAF sites, but not on all the AGE at each site. Less prevalent yet were the *Aspergillus* (Kadena and Andersen only), *Nigrospora* (Kaden and Kunsan only), and *Penicillium* (all three sites) genera; however, species within these genera are known to be involved with MIC. A Summary of fungal results is presented in Table ES- 2.

**Table ES- 2. Potential MIC-causing Fungi Prevalent Across All PACAF Locations**

Prevalent MIC-causing Fungi across three PACAF Locations	Mechanism Influencing MIC	Substrates Affected by MIC
<i>Exophiala</i>	Degrades wood and organic materials; forms biofilms	Water and Metal
<i>Dothideomycetes</i>	Often found as pathogens, endophytes, or epiphytes of living plants; also degrades cellulose and other complex carbohydrates. Several species are lichens, while others occur as parasites.	Unknown
<i>Aureobasidium</i> <sub>6</sub>	Forms biofilms by extracellular polymeric substances (EPS)	Aluminum alloy
<i>Toxicocladosporium / Cladosporium</i> <sub>7</sub>	Metal-ion binding via fungal mycelia	Carbon steel and aluminum alloy
<i>Aspergillus</i> <sub>8</sub>	Oxalic acid metabolite	Magnesium alloy
<i>Penicillium</i> <sub>8</sub>	Forms biofilms by EPS	Aluminum alloy
<i>Nigrospora</i>	Found on decayed wood	Unknown

Characterization of the PACAF samples collected for this task showed presence of wide varieties of bacteria and fungi with the potential for causing MIC. Therefore, it is important to control the microbe's formation on the coatings substrate as these microbes can contribute to the corrosion of the AGE assets. In-depth discussion on the approach for characterization of MIC and the evaluation results are presented in sections 2.3 and 4.4 of this report.

Commercially available UV stabilizers and anti-microbial additives typically added to coatings to improve weathering performance and reduce or eliminate microbial growth are discussed in section 4.5 of this report. The results were discussed with Hentzen coatings and are in consideration for the modification of the LTPC 6191-61003 for subsequent tasks of this project.

The organization of this report is as follows; Introduction of the Project and Objectives of this task are summarized in Section 1. Details on Battelle's coordination activities to schedule the inspection visits and the approach to data collection and achieving the task objectives are discussed in Section 2. Section 3 summarizes the findings from inspections at each of the three locations and documents the samples collected. Section 4 presents the evaluation results of the samples collected during the inspection visits, and the task conclusions are summarized in Section 5. Multiple Appendices are included along with the report for additional raw data.

## 1.0 Introduction

### 1.1 Background

The United States Air Force (USAF) equipment and assets stationed at the Pacific Air Force (PACAF) locations are exposed to highly corrosive environments resulting in excessive damage of the equipment and costs for its sustainment and upkeep. For the USAF, Battelle is working with Hentzen to modify their Commercial-Off-The-Shelf (COTS) Hentzen (Crosslink) Low Temperature Powder Coatings (LTPC) No. 6191-61003 to improve its resistance to Ultraviolet (UV) light and microbiologically influenced Corrosion (MIC). The intent is to transition to this powder coating initially on the Aircraft Ground Equipment (AGE). As part of the project, Battelle inspected and evaluated the AGE at three PACAF locations for the level of degradation from exposure to UV light and MIC.

### 1.2 Scope and Objectives

This technical report is the deliverable for CDRL A002 of the Phase II (PWS paragraph 4.3). It presents Battelle's findings from inspection and evaluation of the AGE at the three PACAF locations for degradation of the coatings from exposure to UV light and potentially from MIC.

The objectives of the task are to inspect the AGE for coatings degradation, corrosion, and evidence of MIC and to understand the similarities as well as differences in the level of UV and MIC induced degradation of coatings on the AGE at different PACAF locations and finally to report these findings to support subsequent project steps. To achieve this, select samples were taken from the AGE and surrounding areas for the purposes of identifying, characterizing and classifying field-prevalent microorganisms and MIC-related bacteria and fungi. The information was used to identify the UV additives and biocides for modification of the LTPC.

## 2.0 Approach for PACAF AGE Inspection & Evaluation

The following three PACAF locations were identified in the performance work statement (PWS) for the inspection of AGE to meet the objectives of the task;

- Kadena Air Base, Okinawa – 18<sup>th</sup> Equipment Maintenance Squadron (EMS)
- Kunsan Air Base, Gunsan – 8<sup>th</sup> Maintenance Squadron (MXS)
- Andersen Air Force Base, Guam – 36<sup>th</sup> MXS

With the Air Force Program Manager's (AFPM) assistance, Battelle coordinated the visits directly with the maintenance squadrons at the three PACAF locations for the inspection and evaluation of the AGE. Two-day inspection visits were planned for each location and the following visit objectives were communicated in advance with the PACAF locations to effectively use the onsite time:

- Inspect, review, and document the condition of a minimum of five (5) AGE assets degraded by microbially induced corrosion (MIC) and from exposure to UV. Collect delaminated coatings, and soil or swab samples to characterize the prevalent microbes in the region
- Discuss the local environment, concerns relative to corrosion, maintenance challenges and the resulting challenges of the AGE groups/squadrons at each location, and
- To explore potential sites at Kadena and Andersen for installation of at least two (2) test racks for the 18-month beachfront testing.

### 2.1 AGE Inspection and Sample Collection

Battelle inspected the AGE for corrosion and weathering performance of the coatings at all the PACAF locations, and photos were taken of each AGE unit being sampled. MIL-PRF-85285E for polyurethane topcoats for aircraft and AGE states in section 3.7.5 that the specular gloss of the coating should be a minimum of 15 and a maximum of 45 at a 60° angle of incidence. Several gloss readings were taken from

AGE assets at the three PACAF locations with a Gardner micro-TRI-gloss meter set for 60° angle of incidence.

Color measurements and specular gloss measurements were taken from the inspected AGE to determine the level of coatings degradation from environmental effects. The color and gloss readings were analyzed at Battelle for the deleterious effects that the environment has on the CIELAB color coordinates of Fed-STD-595 color 26173 as well as the 60° specular gloss for MIL-PRF- 85285 coating. Coating stack-up film thickness measurements were also collected from these assets and are reported in the results section. Where the coating stack-up was delaminated from the inspected AGE, samples of the coating were removed and will be characterized optically to determine primer and topcoat thicknesses within the stack-up.

Samples were collected from the corroded areas on the inspected AGE assets to characterize the observed corrosion. Prior to taking the sample, gloves were disinfected with isopropyl alcohol wipes and a sterile piece of gauze was moistened with 1X phosphate buffered saline (PBS). The coated surfaces of ferrous and non-ferrous substrates of the AGE were wiped thoroughly either by swabbing back and forth three times or by wiping in an "S" shape both horizontally and vertically during sample collection. The gauze was then placed in a sterile 50 mL conical tube containing 5 mL of PBS, sealed with parafilm, and packed into coolers with ice packs to keep temperatures at around 2-8°C, and shipped to Battelle Columbus, Ohio (BCO). Five (5) samples were taken at each base, and three (3) background samples were taken at each base. Four soil (environmental) samples were taken at Kadena, 2 were taken at Kunsan, and 3 were taken at Andersen.

## 2.2 Evaluation of AGE for Degradation from UV

The harsh weather conditions at these locations can cause a high aesthetic degradation, due to the significant development of chalking. The severe environment can cause light induced degradation of the binder along the pigment interfaces, followed by the dissolution of the polymeric breakdown products in water that propagate the loosening of the pigment. These UV-induced and hydrolytic changes in the surface of the coating film can result in a significant loss of gloss.

The Battelle and Hentzen team is experienced in development of coatings as well as their test and assessment in different environment and on substrates and has a good understanding of the material structure. The MIL-PRF 85285E topcoat in the coatings stackup applied on AGE is a polyurethanes (PU) based acrylic polyol and is prone to degradation from exposure to outdoor environments. Although temperature, moisture, and other weathering elements may contribute to the degradation of the polymeric coatings, the primary cause of PU degradation during outdoor exposure is ultraviolet (UV) light. For that reason, the photodegradation mechanism of PU and its model compounds have been studied extensively. Both aromatic and aliphatic isocyanate-based polyurethanes undergo chain scission by photo-oxidation process. Photo-oxidation is a chemical change that reduces the polymer's molecular weight. As a consequence of this change, the material becomes more brittle, with a reduction in its tensile, impact and elongation strength. Discoloration and loss of surface smoothness accompany photo-oxidation.

To determine degradation from UV exposure, color Lab coordinates were collected from the evaluated AGE equipment at three PACAF locations using a X-Rite model SP64 hand held spectrophotometer. The source used was D65/10°. The baseline L\*a\*b\* coordinates are taken from a color standard for FED-STD-595 26173 measured with the same X-Rite hand held color spectrophotometer. Specular gloss readings were taken with a BYK Gardner micro-TRI- gloss meter. Several gloss readings were taken from various locations on the AGE.

Weather and shortwave radiation which includes visible light and ultraviolet radiation historical data were gathered from meteorological web site [weatherspark.com](http://weatherspark.com) for the three PACAF locations included for inspection of the AGE. This data will be used to interpret results in determining the average monthly

temperature, precipitation and incident shortwave solar energy the panels will experience during the beachfront testing of the candidate coatings. This data is included in section 3 of this report for each PACAF location visited.

## 2.3 Microbiology

Samples received at Battelle were unpacked, checked for damage, and images captured. See Table 1 for receipt inventory and Figure 1 for an example of sample receipt. Samples were then placed into a 2 – 8 °C refrigeration unit until ready for extraction. Battelle developed, proprietary work instructions and test protocols were used for extraction and analysis of the sample and are described briefly in the subsequent sections. After extraction via the method listed in Section 2.3.1.1, content was analyzed via 16S and ITS sequencing and for culturable microbes via standard spread plating.

**Table 1. Sample Receipt Inventory**

Site	Corrosion Samples	Background Samples	Environmental Samples (soil)	Control Samples	Condition
Kadena AB, Japan	6	5	5	1	Good
Kunsan AB, South Korea	5	6	2	1	Good
Andersen AFB, Guam	5	5	4	1	Good



**Figure 1. Example Sample Receipt**

### 2.3.1 Extraction

All samples collected for microbial testing from corroded and non-corroded (*i.e.* background) areas were extracted by vortexing the vials containing the sample and 1X PBS buffer and using a serological pipette or pipettor to press the gauze against the side of vial to manually extract liquid from the gauze. The liquid was removed from the vial and placed into a new vial labeled with the appropriate sample name. Next, 5 mL of 1X PBS was added into the vial containing the gauze, capped, and vortexed for 1 minute. The manual extraction described previously was repeated to remove the extraction buffer from the gauze a second time, and was pooled with the first extraction buffer aliquot. The final volume of buffer from each extraction was recorded. For background samples, after individual extraction, 1 mL aliquots of each background sample were removed and pooled into a new vial for each site. Furthermore, some equipment in the field were swabbed at multiple locations; these samples were combined into one composite sample after each swab was extracted individually.

### 2.3.2 Enumeration

Serial 1:10 dilutions of sample extracts were performed using 1X PBS. Neat, 10<sup>-1</sup>, and 10<sup>-2</sup> dilutions were spread plated on Tryptic Soy Agar (TSA) for aerobic bacteria and Potato Dextrose Agar (PDA) for yeast and mold and incubated at 25 ± 2 °C. Plates were checked daily for growth and observations were recorded. After 24 hours, some plates were overgrown and were replated using 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> dilutions. Other plates had slower growth and were incubated for up to 72 hours. Colony counts were recorded, and CFU/mL calculated. Colonies with similar morphologies were observed, and data compiled; all colonies with similar morphologies that were grown on TSA were further isolated onto TSA to examine if those samples were indeed the same species. TSA plates were removed after isolation and stored at 2-8 °C. PDA plates were placed back into the incubator, right-side up to promote fungal growth. These plates were checked weekly thereafter and were removed from incubation after two weeks.

### 2.3.3 Sequencing and Bioinformatics

#### 2.3.3.1 Sample Extraction

Samples suspended in 1X PBS were extracted using the Qiagen DNeasy PowerLyzer Microbial Kit. Samples went through a series of centrifugation steps and were washed with various buffers to lyse open cells and extract the DNA. An ethanol buffer was used to wash away any extracellular debris, and the purified DNA was suspended in an elution buffer prior to library preparation.

#### 2.3.3.2 Library Preparation and Metagenomics Sequencing

After extraction, library preparation was performed on the sample DNA which included a series of PCR reactions to add adapters, primers, and barcodes to amplify DNA prior to sequencing. These steps allowed 16S primers to bind to the DNA and specifically target the 16S rRNA gene sequence present in bacteria. The same steps were followed a second time, only this time, Internal Transcribed Spacer (ITS) primers were used to amplify a region of the rRNA fungal genome. Once library preparation was complete, samples were analyzed using the Agilent Bioanalyzer and Qubit to ensure concentration requirements were met for sequencing. Samples were sequenced using the Illumina MiSeq and per the developed test protocols.

#### 2.3.3.3 Bioinformatics Methods: 16S and ITS

Sequencing data was received on an external hard drive from the laboratory. The hard drive contained the raw fastq files in gzipped format. These files were transferred to the Battelle High Performance Computing System for analysis.

Raw files were unzipped and then merged using the bioinformatic command line paired read merging tool, FLASH<sub>1</sub> v.1.2.11. Before running the reads through the Basic Local Alignment Search Tool (BLAST), a filter was applied to the merged reads to remove low quality reads using the fastq quality filter of the FASTX Toolkit<sub>2</sub>. All reads that did not pass the fastq quality filter were excluded from further analysis. A plot of the quality for each sample was created using the fastq quality boxplot graph shell script, also part of the FASTX Toolkit. These plots allowed a visual inspection of the average quality for each sample after merging and filtering. All samples were of acceptable quality to move on to Blast analysis.

The merged and quality filtered reads were sent through BLAST v.2.6.0, to identify the organisms in each sample. The 16S samples were analyzed through BLAST using the NCBI 16S database v. June 11, 2017. The ITS samples were analyzed through BLAST using the UNITE<sub>3</sub> fungal ITS database v. January 12, 2017. Post BLAST, additional filtering was used to remove false identifications and low-quality identifications. BLAST results were filtered to remove all results with less than 97% identity over less than 80% of the read length. Following this filter, a filter was applied that is designed to remove false positive identifications. False positive identifications are defined as hits that comprise less than 0.01 percent of the

identifications in the results. False positive hits have too few reads identified as belonging to a given organism to be considered strong identifications.

Two scripts from KronaTools<sup>4</sup> were used to get both the TaxID of all filtered BLAST results (reported in an .xlsx spreadsheet) and to generate an interactive Krona plot for relative abundance visualization of the results. The ktClassifyBLAST script gathers the TaxID for all results when the -s flag (summary) is used. The ktImportBLAST script organizes all the results into a single, interactive plot for visualization and exploration of results.

For each organism identified at greater than 1% of the sample, a quick search through the scientific literature was performed to determine origin or bio-activity for the identified organisms. The R programming and statistical analysis language was used to create heat maps to compare sample community composition in a plot.

## 2.4 Industry Available UV Inhibition Additives and Biocides

The performance of an additive is directly related to its chemical structure and exposure conditions. Therefore, it is important to know the chemical functionality of the additives and their activity during manufacturing process and weathering conditions (UV or microorganisms). Battelle conducted a literature search to identify commercially available additives (biocides and UV inhibitors) using the following databases

- a) SciFinder® (Service provided by American Chemical Society),
- b) United States Patent and Trademark Office (USPTO)
- c) Peer reviewed articles from journals focused on paints and coatings namely Coatings Journal, Progress in Organic Coatings and J. Coatings & Technology
- d) Technical and Safety data sheets from selected commercial sources.
- e) Websites reporting about environmental and safety regulations such as California Pro 65, EPA

We used the information gathered from the literature along with other guiding factors such as LTPC manufacturing process, Environmental regulations, field evaluation of microorganisms and weatherability to down select potential additives. The details are reported in section 4.5

## 3.0 PACAF Site Inspections & Findings

### 3.1 AGE Inspection at Kadena Air Base

The 18<sup>th</sup> EMS at Kadena is split between two teams operating on the north and the south side of the flight line. The north team maintains the AGE for large Cargo, Recon, and re-fueling aircraft while the south side team primarily maintains AGE for Fighters. At Kadena AB Battelle evaluated five AGE assets, three from the south side of the flight line and two from the north side of the flight line. According to the 18<sup>th</sup> EMS team, the AGE on the north side is exposed to a more severe environment due to its proximity to the ocean and more frequent precipitation than that on the south side.

According to weatherspark.com at “Kadena Air Base, the summers are hot, oppressive, wet, and overcast; the winters are cool and mostly clear; and it is windy year-round. Over the course of the



Figure 2. Kadena AB (Source: google maps)

year, the temperature typically varies from 57°F to 89°F and is rarely below 49°F or above 92°F. The total daily incident shortwave solar energy reaching the surface of the ground over a wide area, taking full account of seasonal variations in the length of the day, the elevation of the Sun above the horizon, and absorption by clouds and other atmospheric constituents. Shortwave radiation includes visible light and ultraviolet radiation. The average daily incident shortwave solar energy experiences some seasonal variation over the course of the year. The darker period of the year lasts for 2.4 months, from November 20 to February 2, with an average daily incident shortwave energy per square meter below 3.6 kWh. The brighter period of the year lasts for 3.3 months, from June 17 to September 27, with an average daily incident shortwave energy per square meter above 5.1 kWh".

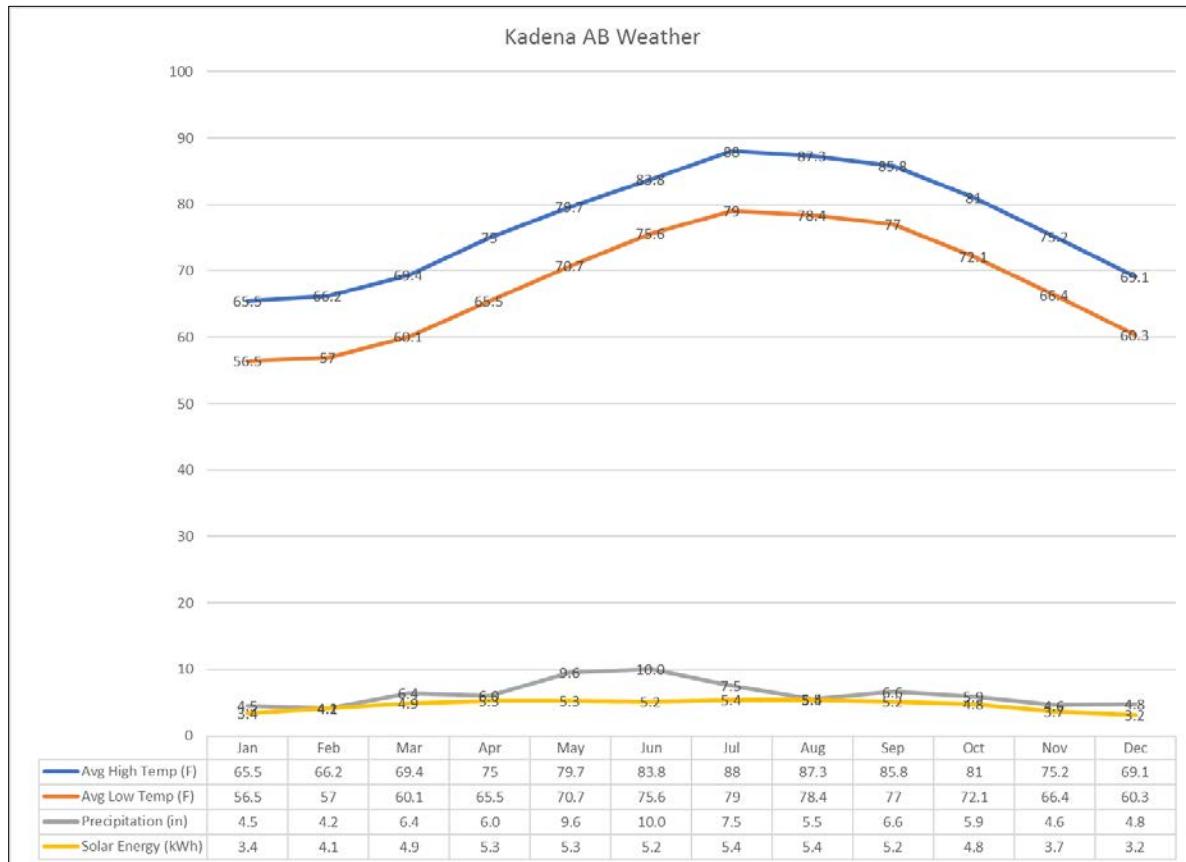


Figure 3 Average annual weather at Kadena AB (Source: <https://weatherspark.com>)

### 3.1.1 Inspection Findings of AGE

Battelle started inspection of the AGE at Kadena on the South side primarily supporting the fighter aircraft. The 18<sup>th</sup> EMS is supported by a contractor operated corrosion repair facility capable of scuff sanding and painting off aircraft components as well as AGE. Because the AGE is subjected to excessive use in harsh open environment and is seldom sent to depots at the CONUS locations for maintenance, this facility is a critical capability for sustainment of the AGE at Kadena. At an interval of about 60 months, corrosion maintenance is performed at this facility on all AGE / assets in accordance with the Tech Order 35-1-3 and coatings are reapplied.

AGE was staged in the parking lot for inspection. To get a representative effect of UV exposure and corrosion effects, Battelle selected five (5) AGE with varying durations from corrosion maintenance ranging from 1 month to close to 60 months.

The following five (5) AGE were inspected at Kadena AB

- C-10 Aircart AU21: 1 month from corrosion maintenance
- Portable Trailer Mounted Load Bank (PTMLB) – LB21: 3 months from corrosion maintenance
- Generator 60 – GT 28: 60 months from corrosion maintenance
- B-1 stand – B1 64 – about 48 months from corrosion maintenance
- Air conditioning and Heating Unit: AA05. This AGE is on the north side supporting large aircraft. Because there are only two of these units, this asset was not scheduled for corrosion maintenance in over 7 years

Many of the corrosion products found on the AGE equipment at Kadena AB were in areas of bolts, leaf springs, under carriage, welds, and crevices between dissimilar metals. These are areas where moisture can collect and in the case of dissimilar metals an electrochemical process can occur in which one metal corrodes preferentially when it is in electrical contact with another, in the presence of an electrolyte. In the areas of welds, the problem arises from the fact that weld metal compositions (which are normally optimized for mechanical properties) tend to be slightly anodic to the parent steel. Therefore, the weld metal corrodes at a higher rate than the parent.

*Significant loss of gloss can be seen between these two assets evaluated at Kadena. The AU-21 aircart was painted 1 month prior to this photo, while the GT28 generator was painted 60 months*



**Figure 4. Photos of AGE in Kadena to show the loss of gloss over time**



*Photos of corrosion products found on AGE at Kadena AB. These are most likely the result of galvanic, crevice or preferential weld corrosion*

Figure 5. Corrosion observed on Inspected AGE at Kadena

### 3.1.2 AGE Inspected at Kadena

Swabs were taken from both corroded and non-corroded areas of the AGE units. Table 2 provides an overview of the sample name, collection location and collection technique used.

Table 2. AGE Inspected at Kadena

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location
J-AU21	Leaf Spring of aircart	Sample was taken by wiping PBS soaked gauze back and forth on three leaflets.	Non-corroded area of aircart
J-LB25	Leaf Spring and left side of generator	Sample was taken by wiping PBS soaked gauze back and forth on three leaflets and	Non-corroded area on left side of AGE

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location
		wiping up and down 3 times left side where CPC was sprayed	
J-GT28	Left side of break linkage and leaf frame on front of generator	Samples taken by swabbing PBS soaked gauze back and forth three times.	Non-corroded area of generator
J-B164	Front step and bar on the back end of B1 stand	Samples taken by swabbing PBS soaked gauze back and forth three times.	Sample taken from non-corroded area of AGE
J-AA05 & J-AA05.2	Lower back right corner and from door on the right side. (J-AA05.2) piece of corroded material.	Samples taken by swabbing PBS soaked gauze back and forth on three leaflets. back and forth three times.	Sample taken from non-corroded area of AGE
Note: All samples from the non-corroded areas were taken by swabbing PBS soaked gauze horizontally and vertically in "S" format			

### 3.1.3 Beachfront Testing Site

While at Kadena, Battelle also worked with the 18<sup>th</sup> EMS to explore location for the 18-month beachfront testing site. Three potential locations were evaluated for southern exposure, proximity to the oceanfront, contractor access to the test site, safety during extreme weather conditions, and other factors of relevance to the location.

Battelle plans to install two exposure racks to mount test coupons for the beachfront testing task. These racks will require about 350 ft<sup>2</sup> – 400 ft<sup>2</sup> in area. During this task, Battelle's subcontractors will periodically evaluate the test coupons each quarter and send a set of coupons back to Battelle for evaluation.

### 3.2 AGE Inspection at Kunsan AB



Figure 6. Kunsan AB (Source: google maps)

According to [weatherspark.com](http://weatherspark.com) "in Gunsan, the summers are warm, muggy, and wet; the winters are very cold, dry, and windy; and it is partly cloudy year-round. Over the course of the year, the temperature typically varies from 24°F to 87°F and is rarely below 15°F or above 92°F. The average daily incident shortwave solar energy experiences significant seasonal variation over the course of the year. The brighter period of the year lasts for 3.9 months, from April 15 to August 12, with an average daily incident shortwave energy per square meter above 5.7 kWh. The darker period of the year lasts for 2.8 months, from November 10 to February 5, with an average daily incident shortwave energy per square meter below 3.1 kWh".

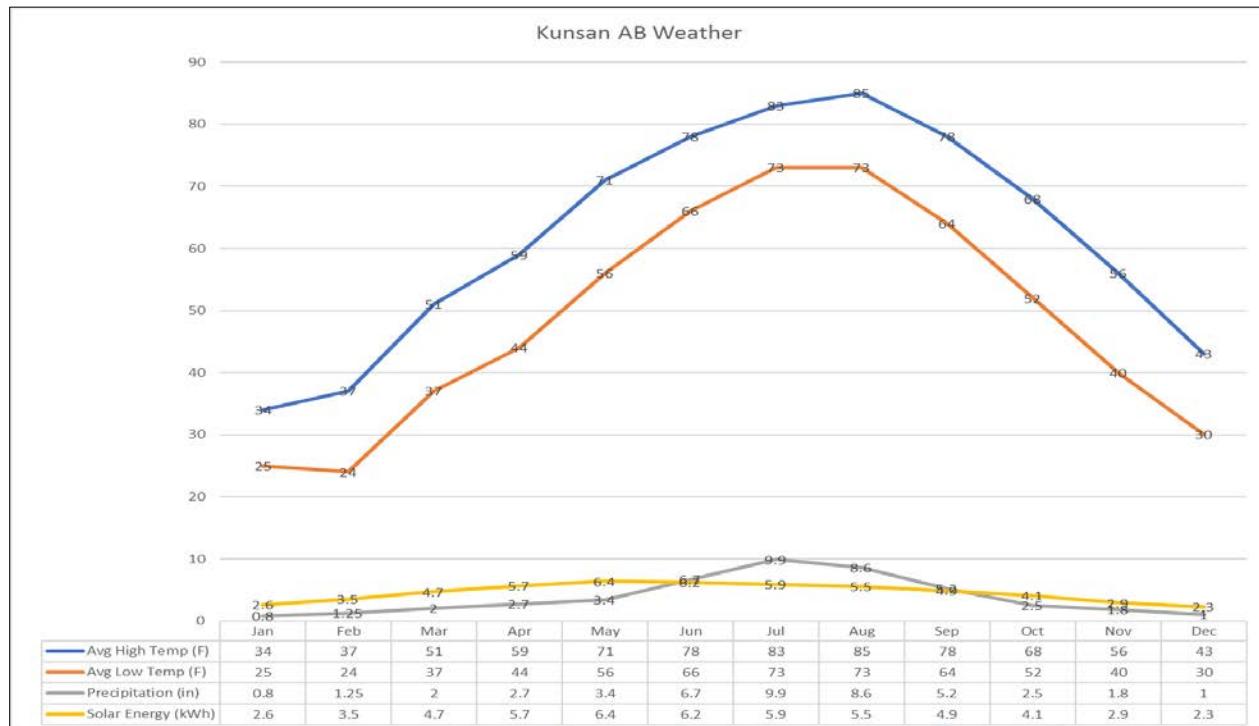


Figure 7 Average annual weather at Kunsan AB (Source: <https://weatherspark.com>)

### 3.2.1 Inspection Findings of AGE

The AGE at Kunsan is maintained by the 8<sup>th</sup> MXS. The assets were similar to those at Kadena. A B4 stand at Kunsan has been with the 8<sup>th</sup> MXS since 1989. Kunsan also has a corrosion repair facility equipped with abrasive blast media and painting capabilities. Records showed that the B4 stand was painted in 2007 and then again in 2014. While several AGE / assets were inspected, samples for characterization of corrosion effects were collected from the following five (5) AGE selected based on the duration from the previous corrosion maintenance/painting.

- Dash 60 Turbine Generator: TG-27; Fresh received from the corrosion repair
- Dash 95: TC-21; Old DRMO, arrived at Kunsan 2001
- 7000 lb Bomb lift; MH10 Arrived at Kunsan 2011; CAT3 and never been painted
- C1-12 stand; No additional information available
- Load Bank LB-04 Arrived at Kunsan 2015 CAT 1



*Example of probable crevice corrosion or corrosion due to compromised coating seen on the undercarriage of AGE at Kunsan AB*

**Figure 8. AGE Inspection Findings at Kunsan AB**



(a)



(b)

*(a) Example of Possible example of MIC from hydraulic oil. (b) Edge effects seen on the fairing of a Dash 95 TC 21 at Kunsan AB. Corrosion peeking through paint at corners is likely a result of edge effects and the coating naturally being thinner there, so it is the first spot to form pores and thus lead to corrosion*

**Figure 9. Additional AGE Inspections at Kunsan**

### 3.2.2 AGE Inspected at Kunsan

Swabs were taken from both corroded and non-corroded areas of the AGE units. Table 3 provides an overview of the sample name, collection location and collection technique used.

Table 3. AGE Inspected at Kunsan

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location	Sample Technique
K-TG27	Top of turbine generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically	Non-corroded area of turbine generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
K-TC21	Inside of AGE and on tow bar	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area of aircart, on both steel and aluminum	Sample taken by swabbing PBS soaked gauze back and forth three times
K-MH10	Bomb lift and front of AGE	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area of bomb lift	Sample taken by swabbing PBS soaked gauze back and forth three times
K-CC12	Under steps of bomb lift	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area of steps of bomb lift	Sample taken by swabbing PBS soaked gauze back and forth three times
K-LB04	Side of load bank	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded side of load bank	Sample taken by swabbing PBS soaked gauze back and forth three times

### 3.3 AGE Inspection at Andersen Air Force Base



Figure 10. Andersen AFB (Source: google maps)

According to weather spark.com at "USAF Andersen Air Force Base, the wet season is hot and overcast, the dry season is warm and partly cloudy, and it is oppressive and windy year round. Over the course of the year, the temperature typically varies from 77°F to 87°F and is rarely below 75°F or above 89°F. The average daily incident shortwave solar energy experiences significant seasonal variation over the course of the year. The brighter period of the year lasts for 2.5 months, from February 28 to May 10, with an average daily incident shortwave energy per square meter above 6.4 kWh. The darker period of the year lasts for 4.0 months, from July 5 to November 6, with an average daily incident shortwave energy per square meter below 4.5 kWh".

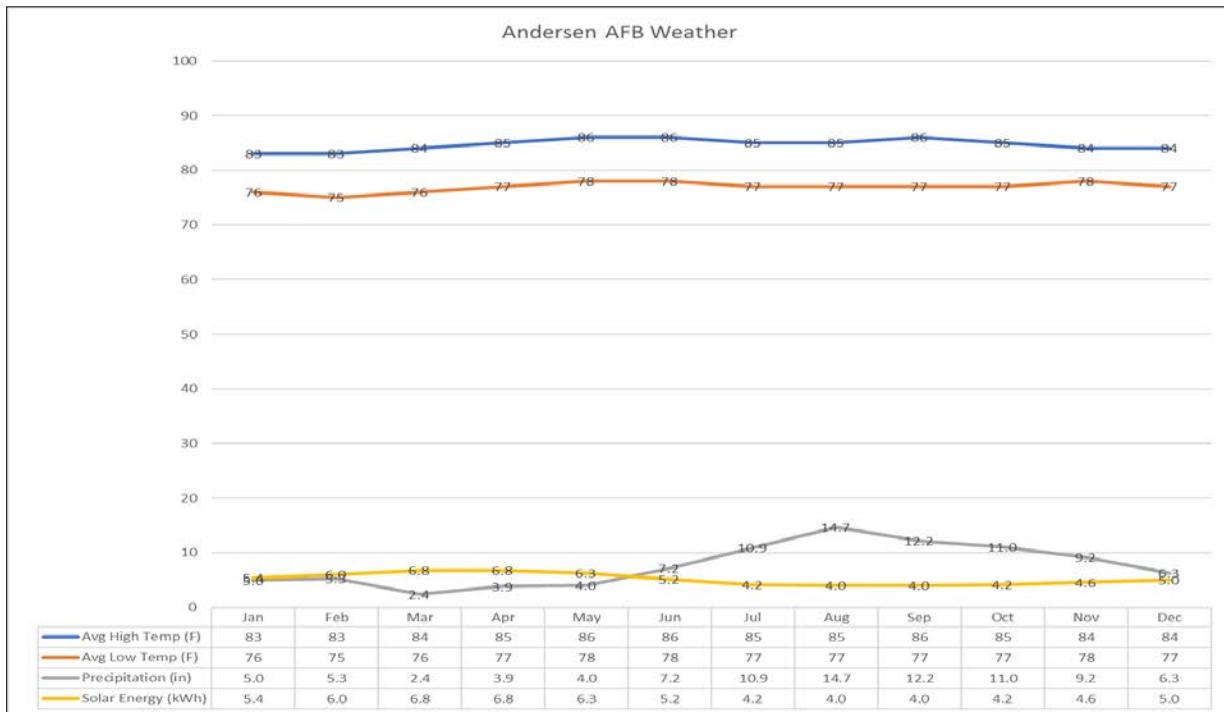


Figure 11 Average annual weather at Andersen AFB (Source: <https://weatherspark.com>)

### 3.3.1 Inspection Findings of AGE

The AGE at Andersen is maintained by the 36 MXG/MXMG and was similar in type and function as the AGE found at Kadena and Kunsan. AGE at Andersen is refurbished just off base by Milspray. This facility has the capability to blast and paint. The AGE at Andersen selected varied in time from last refurbishment from 1 month to 60 months with one asset that was still green in color. Three of the assets were staged outside and two of the assets the Dash 86 power generator PC70 and the 809 generator B8W4 were in the maintenance bay. Evaluations of corrosion and samples were taken from the following.

- MJ-1C bomb lift: MJW6 painted 1 month previously
- Dash 86 generator: PC03 refurbished 1 year ago 9/29/16
- 809 generator: B8W4 green in color, never painted
- Dash 86 generator: PC70 5 years since last painted (Nov 2012)
- B5 stand: B597 no additional information available

Figure 12 shows the striking difference in color and gloss from UV degradation on the bomb lift painted a month ago and the Dash 86 generators painted five year ago and Figure 13 shows corrosion damage on the inspected AGE at Andersen AFB.



Figure 12. UV degradation of coatings at Andersen AFB resulting in loss of gloss

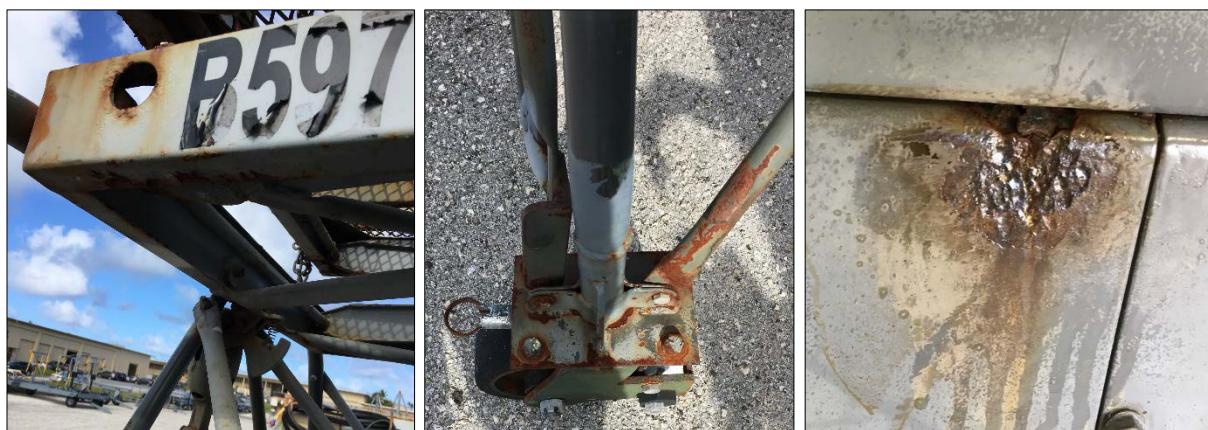


Figure 13 Corrosion types observed on the AGE at Andersen AFB

### 3.3.2 AGE Inspected at Andersen

Swabs were taken from both corroded and non-corroded areas of the AGE units. Table 4 provides an overview of the sample name, collection location and collection technique used.

**Table 4. AGE Inspected at Andersen**

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location	Sample Technique
A-MJW6	Front and side of bomb lift	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area on front of bomb lift	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-PC03	Side of power generator	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded steel area on front of power generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-B8W4	Side, back, and top of generator	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area on generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-PC70	Side of power generator	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded steel area on power generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-B597	Bottom, side and top of B5 stand	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area on B5 stand	Sample taken by swabbing PBS soaked gauze back and forth three times

## 4.0 PACAF AGE Evaluation Results

### 4.1 Impact of UV on AGE Coatings Gloss

Loss of surface gloss and fading is a typical response of a coating due to degradation via UV radiation absorption. Subjectively there is a general reduction in shine, and the surface starts to look chalky. This commonly accepted phenomenon can be observed with any exterior coating. It may be more pronounced in some locations versus others, but the overall trend should be the same.

Figure 14 presents the trend observed on the inspected AGE in gloss reduction through pictures of the AGE at the three PACAF locations.



AU21 Kadena 1 month Gloss = 30.13



LB25 Kadena 3 months Gloss = 9.47



PC70 Andersen 60 months Gloss = 4.47



GT28 Kadena 60 months Gloss = 2.37



TC21 Kunsan 192 months Gloss = 1.53



**Figure 14 UV degradation over time of AGE coatings at the three PACAF Locations**

The gloss readings recorded on AGE at all the three PACAF locations are presented in Table 5.

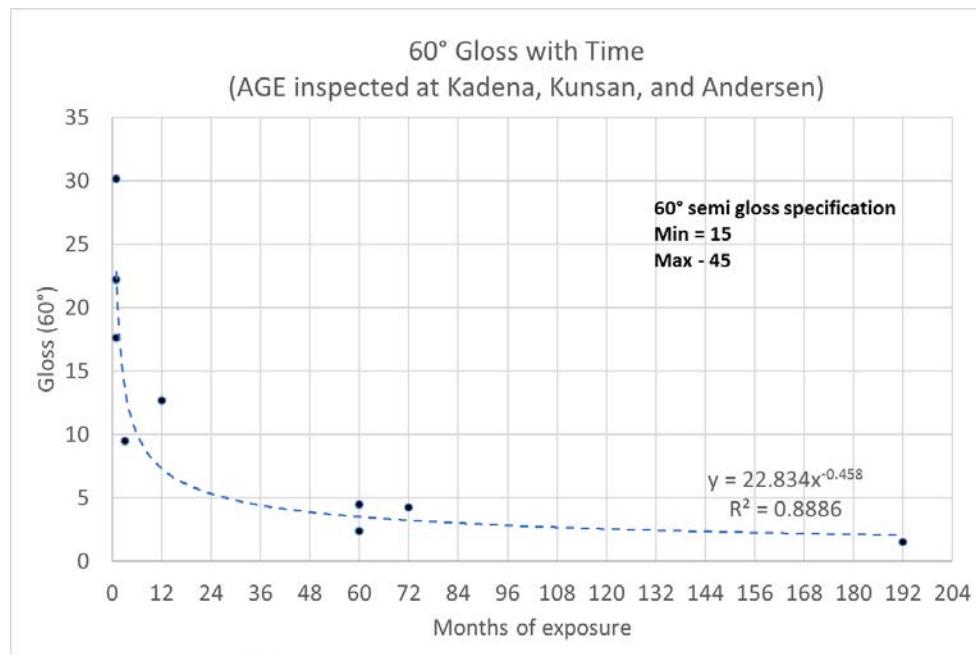
**Table 5 Average gloss readings taken from PACAF weathered AGE**

Location	Equipment Nomenclature	Unit Serial #	Avg. 60° gloss reading	Months since last recorded painting
Kadena South	C10 aircart	AU 21	30.13	1
Kadena South	generator	LB 25	9.47	3
Kadena South	generator	GT 28	2.37	60
Kadena North	B1 stand	B1 64	7.33	unknown
Kadena North	AC + Heat unit	AA 05	1.55	unknown
Kunsan	Dash 60	TG 27	17.63	0*
Kunsan	Dash 95	TC 21	1.53	192
Kunsan	Bomb lift	MH 10	4.23	72
Kunsan	C1 stand	C1 12	28.42	unknown
Kunsan	Load bank	LB 04	4.06	unknown
Andersen	bomblift	MJW6	22.17	1
Andersen	Dash 86	PC-03	23.17	12
Andersen	809 generator	B8W4	14.77	unknown**
Andersen	Dash 86	PC-70	4.47	60
Andersen	B5 stand	B5-97	1.80	unknown

\*Unit still tacky, not completely cured

\*\*Unit was green in color

Assets in which the maturity of the coating could be verified through records were plotted for the 60° gloss readings versus time. This plot included three assets from each of the three PACAF locations. In Figure 16 below we see a dramatic drop in gloss, falling below the minimum specification as soon as three months.

**Figure 15. Plot of 60° gloss readings vs. time**

## 4.2 Impact of UV on AGE Coatings Color

The Air Force's technical order 35-1-3 states in section 3.5 that the standard color for all flight line support equipment is gray 26173 FED-STD-595, MIL-PRF-85285.

Several color readings were taken from PACAF AGE assets with a X-Rite model SP64 hand held spectrophotometer. The source used was D65/10°. The CIELAB coordinates were evaluated to see the effects on the topcoat color of AGE assets located in the PACAF region. The  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$  and  $\Delta E$  were calculated for each of the readings and an average delta is shown in table 6. If you refer to the diagram of the CIELAB color space in Figure 17 you will see that positive (+ve) delta ( $\Delta$ ) correlates to a color reading becoming lighter while a negative (-ve)  $\Delta$  would mean the color is darkening. A positive  $\Delta a$  would mean the color is moving to the red spectrum while a negative  $\Delta a$  shows a move toward the green. A positive move in the  $\Delta b$  shows the color is yellowing and a negative  $\Delta b$  shows the color moving toward the blue spectrum. The  $\Delta E$  deals with how the human eye perceives changes and a general rule of thumb is that the untrained human eye cannot perceive color changes that are less than 1  $\Delta E$ . A look at the  $\Delta L$ ,  $\Delta a$  and  $\Delta b$  shows a general lightening, reddening and yellowing of the polyurethane topcoat on assets that have been sitting in the PACAF environment for any extended period. This is observed by the  $\Delta L$  number moving in a positive direction as well as the  $\Delta a$  and  $\Delta b$  becoming positive in the Lab color space in the Figure 17. Table 6 presents the recorded color coordinates from the AGE inspected at the three PACAF locations.

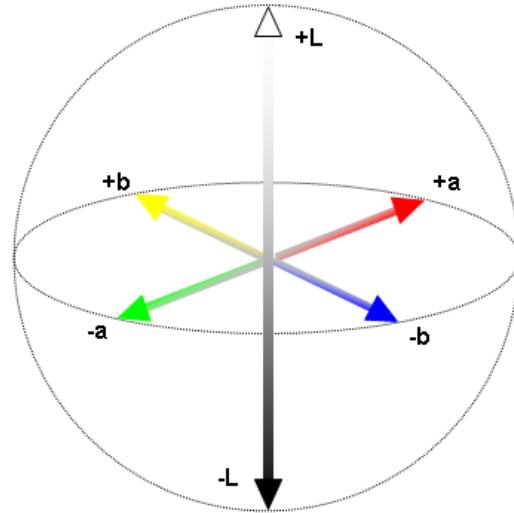


Figure 16 CIELAB color space

Table 6 Average  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$  and  $\Delta E$  color coordinates from AGE in PACAF locations

Location	Equipment nomenclature	Unit Serial #	$\Delta L$	$\Delta a$	$\Delta b$	$\Delta E$	Months since last recorded painting
Kadena S	C10 aircart	Au 21	0.47	0.51	0.44	0.88	1
Kadena S	generator	LB 25	-2.40	0.57	0.76	2.60	3
Kadena S	generator	GT 28	5.42	0.46	1.61	7.52	60
Kadena N	B1 stand	B1 64	-1.07	0.66	0.66	2.33	unknown
Kadena N	AC + Heat unit	AA 05	9.32	0.33	0.42	9.33	unknown
Kunsan	Dash 60	TG-27	-1.95	0.71	0.33	2.11	0*
Kunsan	Dash 95	TC 21	2.44	0.63	2.11	4.95	192
Kunsan	Bomb lift	MH 10	7.56	-0.14	-0.16	7.59	72
Kunsan	C1 stand	C1 12	-2.11	0.59	2.50	3.50	unknown
Kunsan	Load bank	LB 04	6.74	-0.27	-0.85	6.84	unknown
Andersen	Bomb lift	MJW6	0.00	0.36	-0.72	0.81	1
Andersen	Dash 86	PC 03	0.72	0.02	0.11	1.04	12
Andersen	generator	B8W4	-19.12	0.28	8.55	20.96	Unknown**
Andersen	Dash 86	PC 70	5.06	0.25	1.18	5.21	60
Andersen	B1 stand	B1 97	6.63	0.43	1.26	6.81	unknown

\*Unit still tacky, not completely cured

\*\*Unit green in color

### 4.3 Evaluation of Coating Stack-up and Film Thicknesses

Many film thickness measurements were taken on the various AGE assets at various locations with a DeFelsko Positector 6000 coating thickness gage. The results of these film thickness readings can be found in Appendix B. The film thicknesses varied significantly because during the corrosion repair process the AGE is only sanded down to the base metal in areas showing corrosion, and over time the film build can be quite significant. Where the coating stack-up was peeling away from the assets substrate, samples of the coating were removed and were characterized optically to determine primer and topcoat thicknesses within the stack-up. Energy-dispersive X-ray spectroscopy (EDS), is an analytical technique used for the elemental analysis or chemical characterization of a sample. EDS was used to determine the elements in each film layer so it could be identified. Figure 17, Figure 18, and Figure 19 present the evaluation of the corroded samples from the three PACAF locations.

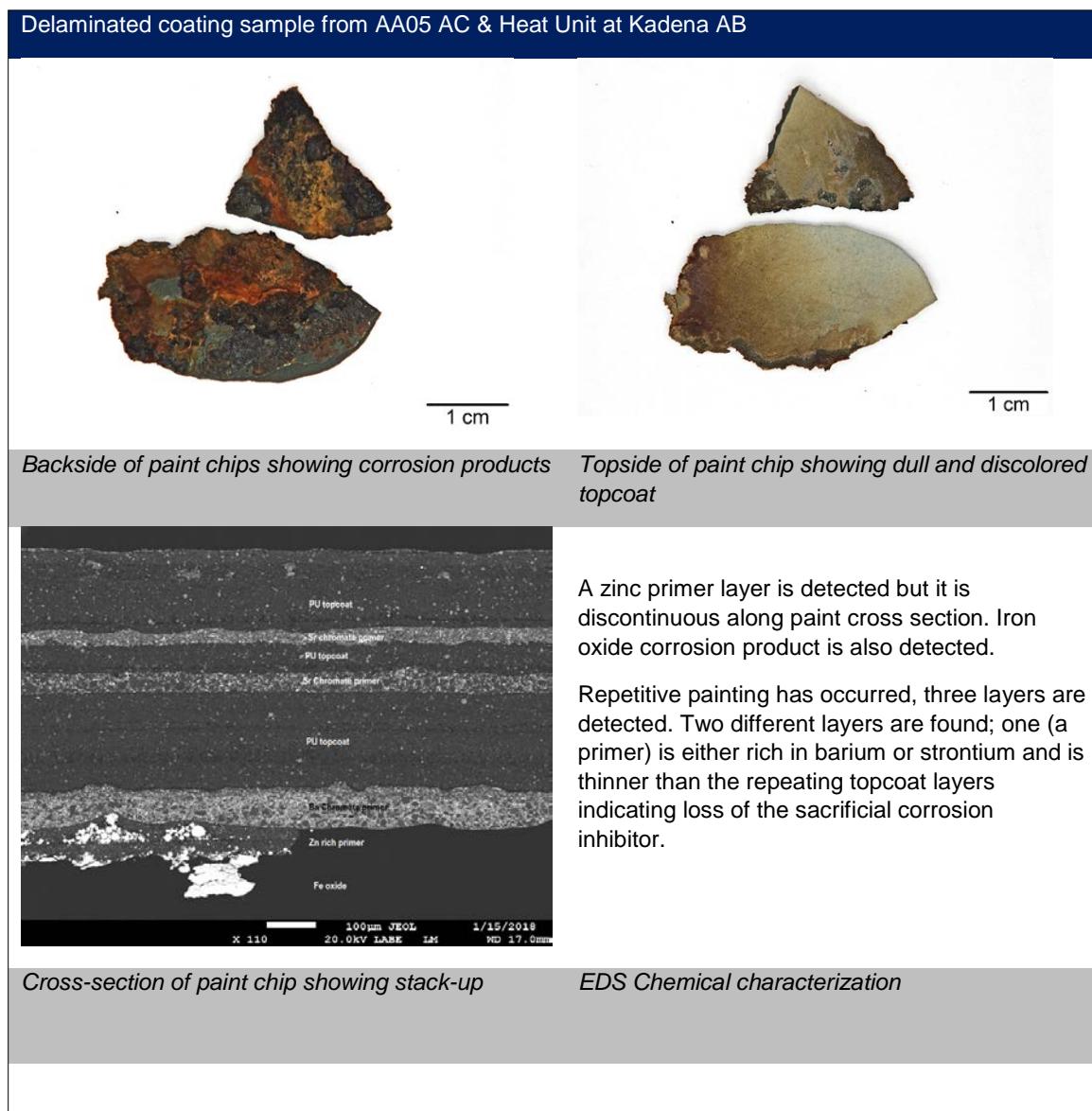
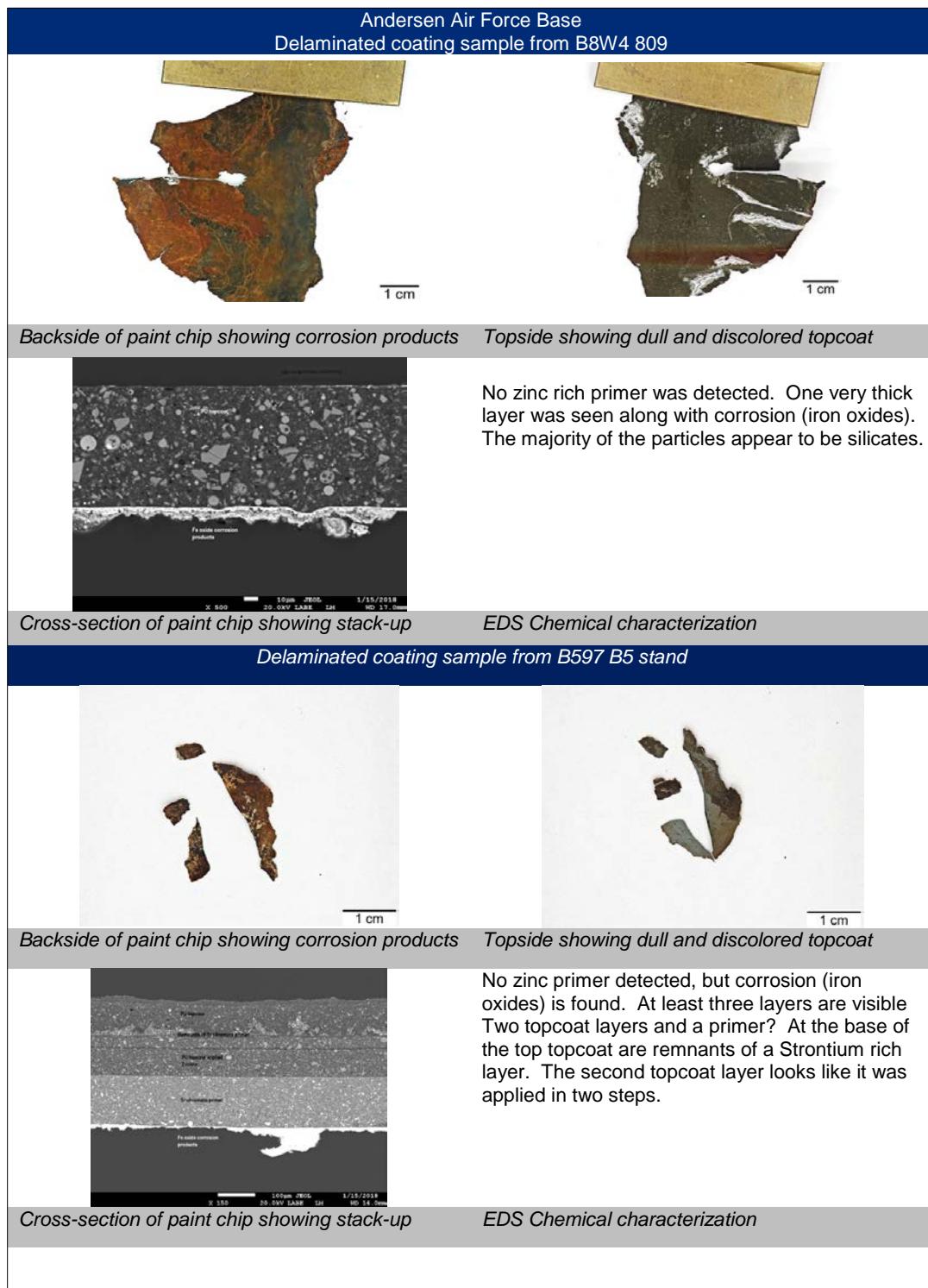
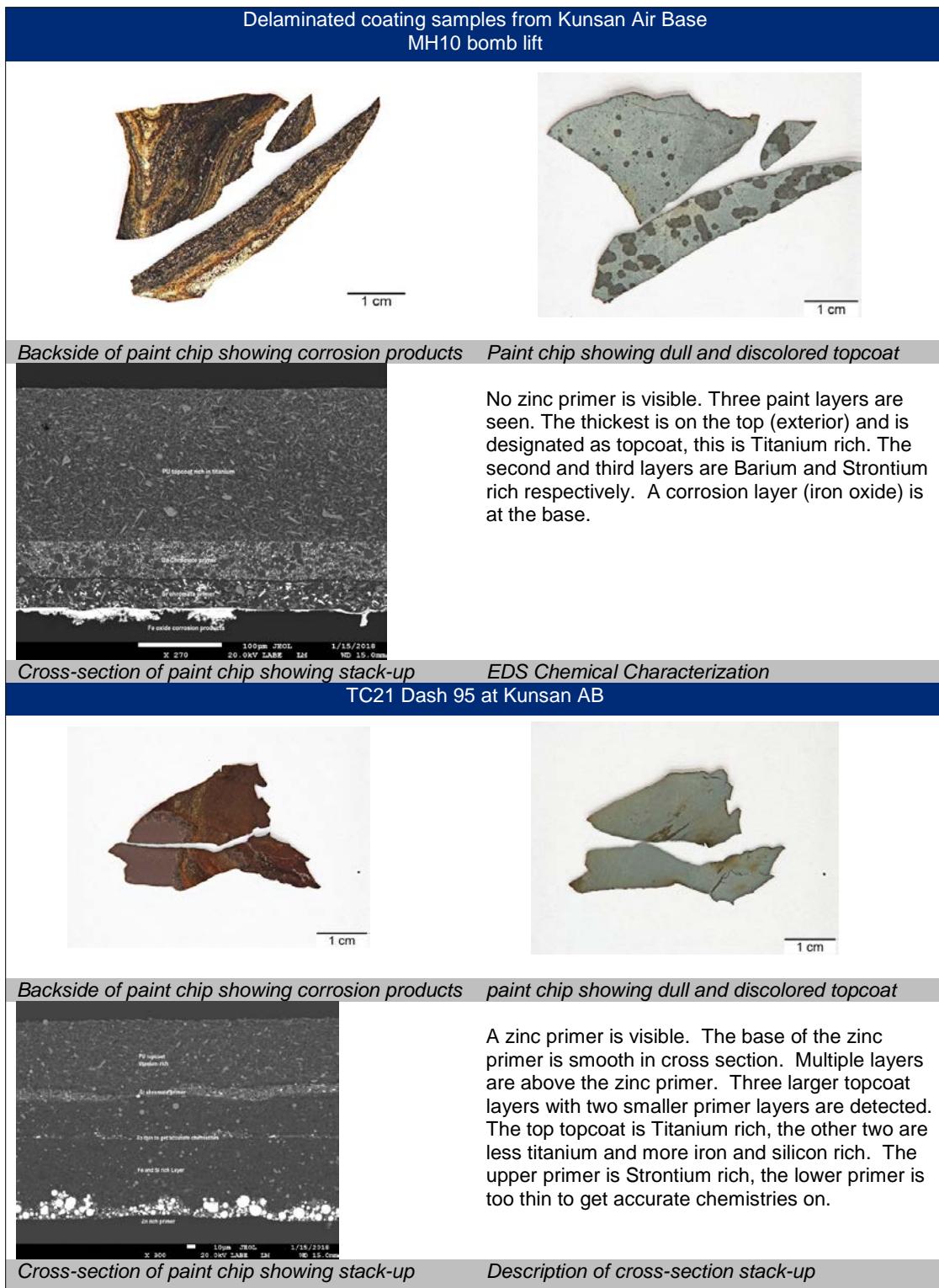


Figure 17. Evaluation of Corroded Coatings Sample from Kadena AB



**Figure 18. Evaluation of Corroded Coatings Sample from Kunsan AB**



**Figure 19. Evaluation of Corroded Coatings Sample from Andersen AFB**

## 4.4 Microbiology Results

The first 24 hours of growth on TSA and PDA plates resulted in overgrown plates for some samples, which led to further dilutions and re-plating ( $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ). Other plates had organisms that grew slower, requiring incubation for up to 72 hours. Counts were similar on TSA and PDA, with the enumeration results from samples plated on both PDA and TSA shown in Table 7. Overall, there were no major fluctuations in the enumerations from site to site, with an approximate range of  $10^2$  CFU/mL to  $10^6$  CFU/mL for Kadena AB,  $10^2$  CFU/mL to  $10^4$  CFU/mL for Kunsan AB, and  $10^3$  CFU/mL to  $10^4$  CFU/mL for Andersen AFB. This range shows that Kadena AB had the largest sample to sample variability, while Andersen AFB had the least. Enumerations from non-corroded areas of the AGE were also equivalent in number to the AGE enumerations, although the background taken at Andersen AFB was ~2 logs lower than the backgrounds from Kadena and Kunsan AB. This is expected as bacteria and fungi can be found essentially everywhere and in equivalent amounts.

**Table 7. Bacterial and Fungal Enumeration Results**

Site	Sample ID	Avg. CFU/mL (PDA)	Avg. CFU/mL (TSA)
Kadena AB, Japan	J-AU21	6.60E+02	6.07E+02
	J-LB25	3.07E+05	2.87E+05
	J-GT28	3.06E+04	1.41E+04
	J-B164	5.87E+05	6.17E+05
	J-AA05	1.21E+06	1.42E+06
	*J-AA05_C	1.02E+04	1.40E+04
	J-BKGRD	1.25E+05	1.12E+05
Kunsan AB, South Korea	K-TG27	4.07E+04	9.73E+04
	K-TC21	1.53E+04	1.55E+04
	K-MH10	3.77E+02	8.27E+02
	K-LB04	9.10E+02	1.01E+03
	K-C112	**TNTC	1.30E+03
	K-BKGRD	5.93E+04	6.13E+05
Andersen AFB, Guam	G-MJW6	1.81E+04	7.31E+04
	G-PC03	3.74E+04	5.59E+04
	G-B8W4	2.07E+04	2.37E+04
	G-PC70	1.60E+04	6.50E+04
	G-B597	4.69E+04	7.69E+04
	G-BKGRD	6.70E+03	6.17E+03

\*Sample= Piece of chipped coating, not gauze.

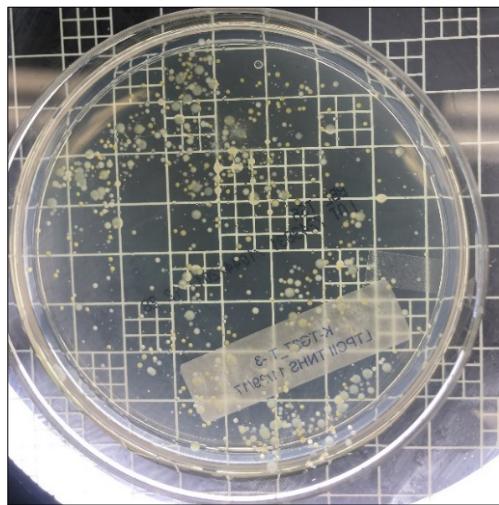
\*\*TNTC= Too numerous to count; plate overgrew after the 24-hour checkpoint

Table 8 describes the bacterial morphologies observed on the TSA plates prior to colony isolation. Figure 18 and Figure 21 show two samples plated on TSA after incubation for 24 hours and 48 hours, respectively. All colonies with similar morphologies on TSA were further isolated onto TSA. Further examination of bacterial morphology on the isolated TSA plates was undertaken (see Figure 22 for an example) and Table 8 updated where possible. No further action past that was undertaken as the

microbiology portion at this point was for informational purposes and the sequencing data provided the details needed for identification (Section 4.4.1).

Table 8. TSA Pre-Isolation Morphology Observations

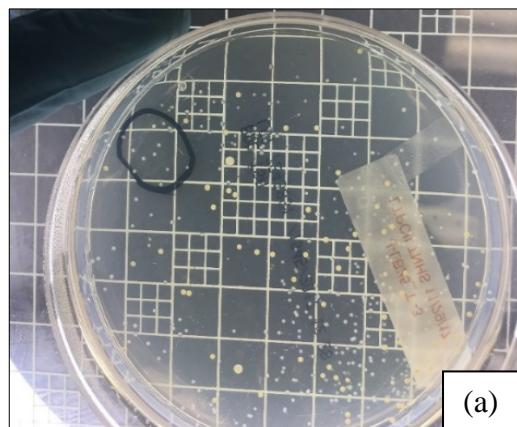
Morphology Label	Morphology Description on TSA	Samples Displaying Morphology
Morphology A	Pinpoint/small, white/off-white, transparent, round/raised/convex, entire	All Plates at All Sites
Morphology B	small, round, raised, entire, shiny, wet	J-B164, J-AA05_C, J-BKGRD, K-TC21, K-NH10, K-LB04, K-C112, K-BKGRD, G-MJW6, G-B8W4, G-PC70, G-B597, G-BKGRD, J-AA05, K-TG27
Morphology C	small-medium, yellow, raised shiny, round	J-AU21, J-LB25, J-GT28, J-AA05_C, K-TC21, K-MH10, K-LB04, K-C112, G-MJW6, G-B8W4, G-PC70, G-B597, J-AA05
Morphology D	white, rhizoid	J-AU21, J-GT28
Morphology E	filamentous, green, raised	J-AU21, K-C112
Morphology F	small, pink, raised round	G-BKGRD, K-LB04, K-TC21, G-B597
Morphology G	yellow/white/filamentous, raised	J-GT28, G-MJW6, G-PC03
Morphology H	yellow/white, wrinkle, craterform	J-AA05_C, G-PC70, G-B597
Morphology I	flat, irregular, medium, clear, yellow	K-TC21
Morphology J	medium, dark orange, raised, round	K-TC21
Morphology K	white, umbonate	G-PC70
Morphology L	yellow, flat, shiny	G-PC70
Morphology M	Small - medium, yellow center, cone, shiny, edge clear/off-white	J-AA05



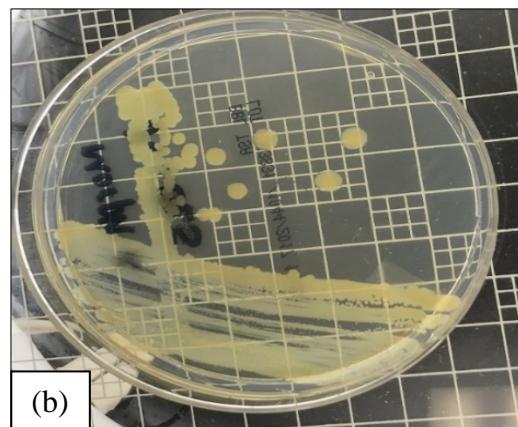
**Figure 20. K-TG27 on TSA after 24 hours at  $25 \pm 2$  °C**



**Figure 21. J-GT28 on TSA after 48 hours at  $25 \pm 2$  °C**



**(a)**



**(b)**

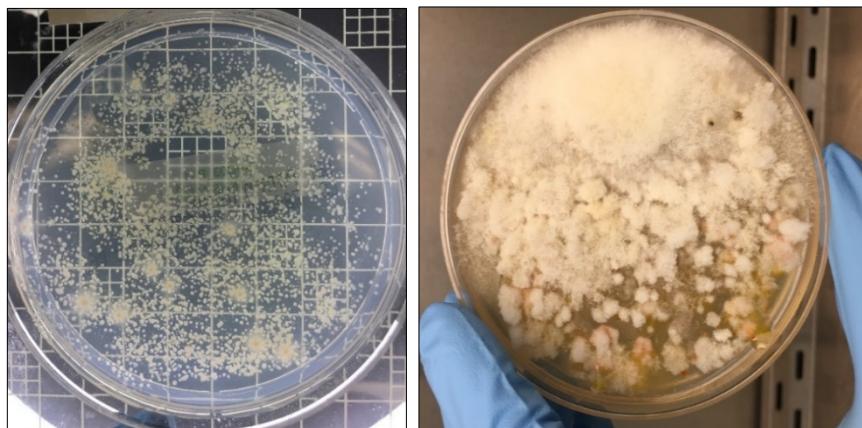
**Figure 22. Morphology A, J-LB25 Colony Selection for Before (a) and After (b) Isolation**

Table 9 describes the fungal morphologies observed on the plates. No isolations were performed on PDA since sequencing was performed also (Section 4.4.1). See Figure 23 for an example of fungal growth observed. Fungal growth was especially prevalent on sample J-AA05\_C, the sample from Kadena AB that was a piece of chipped corroded coating (Figure 24) and was presented in Figure 17 under section 4.3.

Samples collected showed abundant bacterial and fungal growth. Most of the bacterial growth had foul odors, indicating sulfate reducing bacteria (SRB) may be present, which can lead to MIC; as well as other bacteria and fungi. See Section 4.4.1 for the discussion on sequencing results and a more in-depth data analysis as to which organisms are prevalent across all sites.

**Table 9. PDA Morphology Descriptions**

Fungal Morphology Description	Samples Displaying Organism	Possible Microbe
Large, white, rhizoid, cloud-like	G-B8W4, J-GT28, G-PC03, K-TG27, J-AA05_C, J-AU21	<i>Microsporum, Cunninghamella</i>
Black, medium to large, shiny, wet	G-B8W4, J-GT28, G-PC70, K-TC21, J-AA05, J-AA05_C, J-AU21, J-LB25, K-MH10, K-C112, K-LB04, G-BKGRD, K-BKGRD, J-BKGRD	<i>Aspergillus</i>
Yellow, medium, rhizoid	G-B8W4	<i>Microsporum</i>
Large, white rhizoid, red wet under white rhizoid	G-B8W4	<i>Microsporum, Trichophyton, Fusarium</i>
gray, medium, velvet-like	G-B8W4, G-B597, G-BKGRD, J-AA05_C, J-B164, J-LB25	<i>Cladophialophora, Chromoblastomycosis, Aspergillus</i>
Small – medium, white, filamentous	G-B597, G-MJW6	<i>Fusarium, Cladosporium</i>

**Figure 24. G-PC03 on PDA Before and After Fungal Growth Promotion****Figure 23. J-AA05\_C PDA plates**

#### 4.4.1 16S and ITS Metagenomics Sequencing Results

Sequencing data showed a number of microbial communities present in each sample. Due to the large amount of data produced, approximately the top 90% of organisms present in each sample were analyzed. These organisms are presented in Table 10 - Table 12. The tables show bacterial DNA detected on each AGE, and bacterial genuses common across AGE are color coded (e.g. all *Sphingomonas* are highlighted in orange). These color codes are carried across tables so that it can be determined which bacterial genuses were also found on AGE across sites. Common soil microbes found on the AGE, not considered to be contributors to MIC, are highlighted in grey. In one case, in Table 10 (Sample J-B164), some common (and uncommon) pathogenic organisms were observed during sequence analysis (i.e. *Salmonella enterica*, *Trabulsiella guamensis*, *Brevundimonas vesicularis*, and *Salmonella bongori*) and were omitted from further analyses. These organisms are highlighted in grey. After thorough analyses of Table 10 - Table 12, the top set of five bacterial genuses most likely contributing to the MIC across all PACAF locations was compiled. *Sphingomonas* were the most prevalent across all of the PACAF locations, followed by *Massilia*. *Pseudomonas/Brevundimonas* was also selected as it was quite prevalent across all PACAF locations and is also known to contribute to MIC, likely due to its ability to form biofilms. While not nearly as prevalent, *Acidovorax/Variovorax* was also selected as it is known to be an acid producer as well as a biofilm producer, and could very likely be a contributor to MIC. Finally, another potential contributor, again while not as prevalent, is *Methylobacterium*. This genus has been shown to be involved in copper pipe corrosion. Table 13 lists the bacterial genuses of most interest.

ITS sequencing data resulted in the same type of data for fungus. These data are shown in Table 15 - Table 17, and again the tables show fungal DNA detected on each AGE, and fungal genuses common across AGE are color coded (e.g. all *Exophiala* are highlighted in orange). These color codes are carried across tables so that it can be determined which fungal genuses were also found on AGE across sites. After thorough analyses of Table 15 - Table 17, the top set of 7 fungal genuses most likely contributing to the MIC across all PACAF locations was compiled (Table 18). The *Exophiala* genus was by far the most abundant within a PACAF site as well as across sites; however, after that the results were more scattered. *Dothideomycetes* were found across the AGE at the Kadena AB, but were not quite as prevalent on the AGE in Kunsan AB and Andersen AFB. *Toxicocladosporium/Cladosporium* and *Aureobasidium* were also found across all the PACAF sites, but not on all the AGE at each site. Less prevalent yet were the *Aspergillus* (Andersen AFB and Kadena AB only), *Nigrospora* (Kadena AB and Kunsan AB only), and *Penicillium* (all three sites) genuses; however, species within these genuses are known to be involved with MIC so they were included in Table 18.

Table 10. Top ~90% of Bacteria Found on AGE at Kadena AB

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
<i>Alkanindiges hongkongensis</i>	<i>Stenotrophomonas maltophilia</i>	<i>Alkanindiges hongkongensis</i>	<i>Pseudomonas soli</i>	<i>Sphingomonas hankookensis</i>	<i>Trabulsiella guamensis</i>
<i>Pseudoxanthomonas spadix</i>	<i>Stenotrophomonas pavani</i>	<i>Acidovorax delafieldii</i>	<i>Sphingomonas hankookensis</i>	<i>Brevundimonas vesicularis</i>	<i>Brevundimonas vesicularis</i>
<i>Sphingomonas paucimobilis</i>	<i>Brevundimonas vesicularis</i>	<i>Noviherbaspirillum psychrotolerans</i>	<i>Pseudomonas punonensis</i>	<i>Pseudomonas stutzeri</i>	<i>Salmonella enterica subsp. enterica serovar Typhimurium</i>
<i>Rhizobium pakistanensis</i>	<i>Sphingomonas kyungheensis</i>	<i>Pseudoxanthomonas mexicana</i>	<i>Sphingomonas dokdonensis</i>	<i>Sphingomonas dokdonensis</i>	<i>Agrobacterium fabrum</i>
	<i>Sphingomonas adhaesiva</i>	<i>Blastocatella fastidiosa</i>	<i>Massilia namcuonensis</i>	<i>Microbacterium chocolatum</i>	<i>Janthinobacterium agaricidamnosum</i>
<i>Massilia suwonensis</i>	<i>Brevundimonas lenta</i>	<i>Novosphingobium barchamii LL02</i>		<i>Microbacterium aurantiacum</i>	<i>Pseudomonas oryzihabitans</i>
<i>Sphingomonas hankookensis</i>	<i>Noviherbaspirillum suwonense</i>	<i>Sphingomonas kyungheensis</i>	<i>Sphingomonas kyungheensis</i>		<i>Sphingobium abikonense</i>
<i>Noviherbaspirillum suwonense</i>	<i>Sphingomonas roseiflava</i>	<i>Roseomonas gillardii</i>	<i>Microbacterium oleivorans</i>		<i>Sphingomonas pituitosa</i>
<i>Paracoccus carotinifaciens</i>	<i>Xenophilus aerolatus</i>	<i>Enterobacter kobei</i>	<i>Bosea robiniae</i>		<i>Acidovorax wautersii</i>
<i>Noviherbaspirillum canariense</i>	<i>Methylobacterium tarhaniae</i>	<i>Pseudoxanthomonas spadix</i>	<i>Spirosoma rigui</i>		<i>Massilia consociata</i>
<i>Sphingomonas paucimobilis</i>	<i>Sphingomonas adhaesiva</i>	<i>Leclercia adecarboxylata</i>	<i>Pseudoxanthomonas mexicana</i>		<i>Salmonella bongori</i>
<i>Sphingomonas gimensis</i>	<i>Tetrasphaera remsis</i>	<i>Massilia oculi</i>	<i>Massilia arvi</i>		<i>Sphingomonas hankookensis</i>
<i>Blastocatella fastidiosa</i>	<i>Sphingomonas kyungheensis</i>	<i>Curtobacterium oceanosedimentum</i>	<i>Sphingomonas aquatilis</i>		<i>Bordetella sp. R-39474</i>
<i>Sphingomonas desiccabilis</i>	<i>Chryseobacterium profundimaris</i>	<i>Rhizobium qilianshanense</i>	<i>Rhizobium cellulosilyticum</i>		<i>Massilia arvi</i>
<i>Methylobacterium phyllostachyos</i>	<i>Sphingomonas ginsengisoli</i>	<i>Rhizobium grahamii</i>	<i>Agrobacterium larrymoorei</i>		<i>Curtobacterium oceanosedimentum</i>
<i>Spirosoma rigui</i>	<i>Acetobacteraceae bacterium WS10</i>	<i>Agrobacterium fabrum</i>			

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
	<i>Sphingomonas dokdonensis</i>	<i>Methylobacterium phyllostachyos</i>			
	<i>Mucilaginibacter litoreus</i>	<i>Methylocapsa sp. NE2</i>			
	<i>Spirosoma sp. MSd3</i>	<i>Microvirga guangxiensis</i>			
	<i>Tersicoccus phoenicis</i>	<i>Flavisolibacter ginsenosidimutans</i>			
	<i>Brevundimonas viscosa</i>	<i>Noviherbaspirillum canariense</i>			
	<i>Methylobacterium tarhaniae</i>	<i>Variovorax defluvii</i>			
	<i>Massilia brevitalea</i>	<i>Methylobacterium soli</i>			
	<i>Microbacterium aerolatum</i>	<i>Alsobacter metallidurans</i>			
	<i>Novosphingobium naphthalenivorans</i>	<i>Sphingomonas dokdonensis</i>			
	<i>Microbacterium assamensis</i>	<i>Azospirillum oryzae</i>			
	<i>Variovorax defluvii</i>	<i>Sphingomonas desiccabilis</i>			
	<i>Rhizobium cellulosilyticum</i>				
	<i>Rhizobium endophyticum CCGE 2052</i>				
	<i>Mesorhizobium mediterraneum</i>				
	<i>Pseudarthrobacter equi</i>				

Table 11. Top ~90% of Bacteria Found on AGE at Kunsan AB

K-TG27	K-TC21	K-MH10	K-LB04	K-C112
<i>Massilia timonae</i>	<i>Sphingomonas</i> sp. DCY91	<i>Brevundimonas vesicularis</i>	<i>Massilia jejuensis</i>	<i>Pseudochrobactrum lubricantis</i>
<i>Pseudomonas abietaniphila</i>	<i>Pseudomonas punonensis</i>	<i>Noviherbaspirillum suwonense</i>	<i>Sphingomonas</i> sp. JM-791	<i>Luteibacter rhizovicinus</i>
<i>Sphingomonas paucimobilis</i>	<i>Noviherbaspirillum suwonense</i>	<i>Rhodococcus corynebacterioides</i>	<i>Pseudomonas oryzihabitans</i>	<i>Variovorax boronicumulans</i> (NBRC 103145)
<i>Brevundimonas vesicularis</i>	<i>Sphingomonas kyungheensis</i>	<i>Sphingomonas hankookensis</i>	<i>Sphingomonas paucimobilis</i>	<i>Brevundimonas vesicularis</i>
<i>Sphingobium rhizovicinum</i>	<i>Ochrobactrum pseudogrignonense</i>	<i>Pseudorhodoferax caeni</i>	<i>Massilia oculi</i>	<i>Bacillus aquimaris</i>
<i>Novosphingobium lindaniclasticum</i> LE124	<i>Sphingobium rhizovicinum</i>	<i>Pigmentiphaga litoralis</i>	<i>Sphingomonas dokdonensis</i>	<i>Okibacterium fritillariae</i>
<i>Noviherbaspirillum suwonense</i>	<i>Exiguobacterium acetylicum</i>	<i>Sphingomonas kyungheensis</i>	<i>Brevundimonas vesicularis</i>	<i>Sphingomonas kyungheensis</i>
<i>Sphingomonas</i> sp. JM-791	<i>Brevundimonas vesicularis</i>	<i>Luteibacter rhizovicinus</i>	<i>Pigmentiphaga litoralis</i>	<i>Aureimonas ureilytica</i> (DSM 18598 = NBRC 106430)
<i>Massilia suwonensis</i>	<i>Sphingomonas kyeonggiensis</i>	<i>Massilia consociata</i>	<i>Massilia tieshanensis</i>	<i>Curtobacterium oceanosedimentum</i>
<i>Roseomonas aerophila</i>	<i>Sphingomonas hankookensis</i>	<i>Massilia putida</i>	<i>Sphingomonas kyungheensis</i>	<i>Rhizobium pakistanensis</i>
<i>Massilia namucuongensis</i>	<i>Paenibacillus taohuashanense</i>	<i>Sphingomonas roseiflava</i>	<i>Massilia varians</i>	<i>Curtobacterium flaccumfaciens</i>
<i>Microbacterium assamensis</i>	<i>Pseudomonas oryzihabitans</i>	<i>Microbacterium ginsengiterrae</i>	<i>Stenotrophomonas pavani</i>	<i>Curtobacterium albidum</i>
<i>Agrobacterium larrymoorei</i>	<i>Exiguobacterium indicum</i>	<i>Sphingomonas yunnanensis</i>	<i>Noviherbaspirillum suwonense</i>	
<i>Rhizobium soli</i> DS-42	<i>Bacillus vireti</i> LMG 21834	<i>Achromobacter animicus</i>	<i>Microbacterium ginsengiterrae</i>	
<i>Rhizobium skieri</i> new license	<i>Sphingomonas roseiflava</i>	<i>Agrobacterium larrymoorei</i>	<i>Sphingomonas roseiflava</i>	
<i>Curtobacterium oceanosedimentum</i>	<i>Sphingomonas dokdonensis</i>	<i>Curtobacterium oceanosedimentum</i>	<i>Paenibacillus pabuli</i>	
<i>Agrobacterium rubi</i>	<i>Methylobacterium phyllostachyos</i>	<i>Rhizobium cellulosilyticum</i>	<i>Massilia suwonensis</i>	

K-TG27	K-TC21	K-MH10	K-LB04	K-C112
	<i>Rhizobium soli</i> DS-42	<i>Pseudarthrobacter equi</i>	<i>Rhizobium cellulosilyticum</i>	
	<i>Pseudarthrobacter phenanthrenivorans</i> <i>Sphe3</i>	<i>Pseudarthrobacter phenanthrenivorans</i> <i>Sphe3</i>	<i>Rhizobium soli</i> DS-42	
	<i>Pseudarthrobacter equi</i>	<i>Curtobacterium flaccumfaciens</i>	<i>Rhizobium grahamii</i>	
	<i>Curtobacterium oceanosedimentum</i>			<i>Curtobacterium oceanosedimentum</i>
	<i>Agrobacterium larrymoorei</i>			

Table 12. Top ~90% of Bacteria Found on AGE at Andersen AFB

G-B8W4	G-PC70	G-B597	G-MJW6	G-PC03
<i>Pseudomonas stutzeri</i>	<i>Pseudomonas stutzeri</i>	<i>Alkanindiges hongkongensis</i>	<i>Sphingomonas kyungheensis</i>	<i>Noviherbaspirillum canariense</i>
<i>Chryseobacterium hagamense</i>	<i>Sphingomonas dokdonensis</i>	<i>Pseudomonas stutzeri</i>	<i>Sphingomonas paucimobilis</i>	<i>Sphingomonas hankookensis</i>
<i>Pedobacter sp. THG-G12</i>	<i>Pseudomonas sp. KMM 9500</i>	<i>Sphingomonas hankookensis</i>	<i>Mycoplana ramosa</i>	<i>Sphingomonas kyungheensis</i>
<i>Massilia consociata</i>	<i>Sphingomonas hankookensis</i>	<i>Pseudarthrobacter equi</i>	<i>Aureimonas phyllosphaerae</i>	<i>Noviherbaspirillum soli</i>
<i>Brevundimonas vesicularis</i>	<i>Sphingomonas kyungheensis</i>	<i>Agrobacterium larrymoorei</i>	<i>Microbacterium assamensis</i>	<i>Sphingomonas adhaesiva</i>
<i>Sphingobacterium kyonggiense</i>	<i>Brevundimonas vesicularis</i>	<i>Sphingomonas kyungheensis</i>	<i>Methylobacterium komagatae</i> (DSM 19563)	<i>Oxalicibacterium solurbis</i>
<i>Devosia lucknowensis</i>	<i>Cellulomonas pakistanensis</i>	<i>Oxalicibacterium solurbis</i>	<i>Methylobacterium pseudosasae</i>	<i>Novosphingobium subterraneum</i>
<i>Brevundimonas olei</i>	<i>Curtobacterium albidum</i>	<i>Noviherbaspirillum canariense</i>	<i>Sphingomonas hankookensis</i>	<i>Sphingomonas kyeonggiensis</i>
<i>Sphingobacterium daejeonense</i>		<i>Rhodococcus cerastii</i>	<i>Aureimonas ureilytica</i> (DSM 18598 = NBRC 106430)	<i>Methylobacterium hispanicum</i>
<i>Massilia suwonensis</i>		<i>Noviherbaspirillum soli</i>	<i>Microbacterium oleivorans</i>	<i>Novosphingobium naphthalenivorans</i>

G-B8W4	G-PC70	G-B597	G-MJW6	G-PC03
<i>Pseudomonas</i> sp. <i>KMM 9500</i>		<i>Sphingomonas adhaesiva</i>	<i>Microbacterium marinum</i>	<i>Microbacterium mitrae</i>
<i>Parapusillimonas granuli</i>		<i>Tersicoccus phoenicis</i>	<i>Massilia timonae</i>	<i>Agrobacterium larrymoorei</i>
<i>Sphingobacterium lactis</i>		<i>Sphingomonas dokdonensis</i>	<i>Brevundimonas vesicularis</i>	
<i>Sphingobium rhizovicinum</i>		<i>Novosphingobium naphthalenivorans</i>	<i>Curtobacterium pusillum</i>	
<i>Shinella zoogloeooides</i>		<i>Curtobacterium oceanosedimentum</i>	<i>Curtobacterium oceanosedimentum</i>	
<i>Chryseobacterium arachidiradicis</i>				
<i>Achromobacter animicus</i>				
<i>Agrobacterium larrymoorei</i>				
<i>Agrobacterium tumefaciens</i>				

**Table 13. Potential MIC-causing Bacteria Prevalent Across All PACAF Locations**

Prevalent Bacteria Across the Three PACAF Locations	Mechanism Influencing MIC	Cell Wall Type
<i>Sphingomonas</i> <sup>1,2</sup>	Degradates alkane, Copper-degrading, Nitrate-reducing	Gram Negative
<i>Acidovorax</i> <sup>3,4</sup> / <i>Variovorax</i> <sup>5</sup>	Biofilm formation (Variovorax), Copper-degrading (Variovorax), Iron-oxidizing (Acidovorax), Nitrate-reducing (Acidovorax)	Gram Negative
<i>Pseudomonas</i> <sup>6,7,8,9</sup> / <i>Brevundimonas</i> <sub>9</sub>	Forms biofilms, Stainless steel corrosion, Copper-degrading, Nitrate-reducing, Carbon Steel degrading	Gram Negative
<i>Massilia</i> <sup>10</sup>	Found in MIC-causing consortia, Copper-degrading	Gram Negative
<i>Methylobacterium</i>	Copper corrosion	Gram Negative

**Table 14. Top ~90% of Fungi Found on AGE at Kadena AB**

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
<i>Cystobasidium minutum</i>	<i>Exophiala xenobiotica</i>	<i>Dothideomycetes</i> sp.; <i>Didymellaceae</i> sp. ZLY-2010	<i>Aureobasidium namibiae</i>	<i>Exophiala xenobiotica</i>	<i>Exophiala oligosperma</i>
<i>Nigrospora oryzae</i>	<i>Dothideomycetes</i> sp.; <i>Didymellaceae</i> sp. ZLY-2010	<i>Exophiala xenobiotica</i>	<i>Cystobasidium benthicum</i>	<i>Aureobasidium namibiae</i>	<i>Exophiala xenobiotica</i>

<sup>1</sup> Pavissich *et al.*, Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. 2010. Journal of Applied Microbiology 109; 771–782.<sup>2</sup> Leigh *et al.*, Microbial communities biostimulated by ethanol during uranium (VI) bioremediation in contaminated sediment as shown by stable isotope probing, 2015. Front. Environ. Sci. Eng. 9(3): 453–464.<sup>3</sup> Wang *et al.*, Effects of disinfectant and biofilm on the corrosion of cast iron pipes in a reclaimed water distribution system. 2012. Water Research 46 (2012) 1070 e1078.<sup>4</sup> Leigh *et al.*, Microbial communities biostimulated by ethanol during uranium (VI) bioremediation in contaminated sediment as shown by stable isotope probing, 2015. Front. Environ. Sci. Eng. 9(3): 453–464.<sup>5</sup> Pavissich *et al.*, Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. 2010. Journal of Applied Microbiology 109; 771–782.<sup>6</sup> Jia R. *et al.*, Anaerobic Corrosion of 304 Stainless Steel Caused by the *Pseudomonas aeruginosa* Biofilm. 2017. Front. Microbiol. 8:2335.<sup>7</sup> Aruliah and Ting, Characterization of Corrosive Bacterial Consortia Isolated from Water in a Cooling Tower. 2014. ISRN Corrosion Volume 2014, Article ID 803219.<sup>8</sup> Pavissich *et al.*, Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. 2010. Journal of Applied Microbiology 109; 771–782.<sup>9</sup> Rajasekar *et al.*, Characterization of corrosive bacterial consortia isolated from petroleum-product-transporting pipelines. 2010. Appl Microbiol Biotechnol. 85:1175–1188<sup>10</sup> Aruliah and Ting, Characterization of Corrosive Bacterial Consortia Isolated from Water in a Cooling Tower. 2014. ISRN Corrosion Volume 2014, Article ID 803219.

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
<i>Toxicocladosporium irritans</i>	<i>Exophiala phaeomuriformis</i>	<i>Resinicium saccharicola</i>	<i>Toxicocladosporium irritans</i>	<i>Toxicocladosporium irritans</i>	<i>Nigrospora oryzae; Nigrospora spaerica</i>
<i>Dothideomycetes sp.; Didymellaceae sp. ZLY-2010</i>	<i>Phialophora chinensis</i>	<i>Marasmius tenuissimus</i>	<i>Dothideomycetes sp.; Didymellaceae sp. ZLY-2010</i>	<i>Nigrospora oryzae; Nigrospora spaerica</i>	<i>Cyphelophora europaea</i>
<i>Cordyceps bassiana; Beauveria bassiana</i>	<i>Exophiala dermatitidis</i>	<i>Gymnopilus dilepis</i>	<i>Chaetothyriales sp</i>	<i>Cystobasidium benthicum</i>	<i>Dothideomycetes sp.; Didymellaceae sp. ZLY-2010</i>
<i>Exophiala xenobiotica</i>		<i>Coniosporium uncinatum</i>	<i>Nigrospora oryzae; Nigrospora spaerica</i>	<i>Dothideomycetes sp.; Didymellaceae sp. ZLY-2010</i>	
<i>Penicillium oxalicum</i>		<i>Diatrypaceae sp</i>	<i>Pestalotiopsis coffeae-arabicae</i>		
<i>Moesziomyces parantarcticus</i>		<i>Ascomycota sp</i>	<i>Exophiala xenobiotica</i>		
		<i>Aplosporella javeedii</i>			
		<i>Eutypella citricola</i>			
		<i>Toxicocladosporium irritans</i>			
		<i>Exophiala oligosperma</i>			
		<i>Ochroconis musae</i>			
		<i>Knufia epidermidis</i>			
		<i>Nigrospora oryzae; Nigrospora spaerica</i>			
		<i>Exophiala dermatitidis</i>			
		<i>Ochroconis globalis</i>			
		<i>Aureobasidium namibiae</i>			
		<i>Entolomataceae sp</i>			
		<i>Ascomycota sp.; fungal sp. E08</i>			
		<i>Hydnochaete japonica</i>			

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
		<i>Agaricus rotalis</i>			
		<i>Aspergillus pulvericola</i>			
		<i>Hymenochaete muroiana</i>			
		<i>Ustilaginaceae sp</i>			

Table 15. Top ~90% of Fungi Found on AGE at Kunsan AB

K-TG27	K-TC21	K-MH10	K-LB04	K-C112
<i>Hypocreales sp</i>	<i>Calyptrozyma sp</i>	<i>Exophiala sp</i>	<i>Chaetothyriales sp</i>	<i>Cystobasidium lysinophilum</i>
<i>Nigrospora oryzae; Nigrospora spaerica</i>	<i>Aureobasidium namibiae</i>	<i>Exophiala heteromorpha</i>	<i>Aureobasidium namibiae</i>	<i>Aureobasidium namibiae</i>
<i>Montagnulaceae sp.; Dothidiomycetes sp.</i>	<i>Sarocladium bactrocephalum</i>	<i>Aureobasidium namibiae</i>	<i>Exophiala sp</i>	<i>Pseudozyma pruni</i>
<i>Sarocladium bactrocephalum</i>	<i>Hypocreales sp</i>	<i>Pleosporales sp</i>	<i>Cryptococcus saitoi</i>	<i>Nigrospora oryzae; Nigrospora spaerica</i>
<i>Penicillium cinerascens</i>	<i>Sporobolomyces carnicolor</i>	<i>Exophiala xenobiotica</i>	<i>Hypocreales sp</i>	<i>Montagnulaceae sp.; Dothidiomycetes sp.</i>
<i>Ustilaginaceae sp</i>	<i>Exophiala xenobiotica</i>		<i>Trichomeriaceae sp</i>	<i>Ascomycota sp</i>
<i>Cladosporium delicatulum</i>	<i>Chaetothyriales sp</i>		<i>Pleosporales sp</i>	<i>Dioszegia zsoldii var. zsoldii</i>
<i>Agaricomycetes sp</i>	<i>Sporobolomyces phaffii</i>		<i>Exophiala xenobiotica</i>	<i>Pleosporales sp</i>

Table 16. Top ~90% of Fungi Found on AGE at Andersen AFB

G-B8W4	G-PC70	G-B597	G-MJW6	G-PC03
<i>Hortaea werneckii</i>	<i>Aureobasidium namibiae</i>	<i>Toxicocladosporium irritans</i>	<i>Dothideomycetes sp</i>	<i>Exophiala xenobiotica</i>
<i>Aspergillus penicillioides</i>	<i>Ischnoderma resinosum</i>	<i>Exophiala xenobiotica</i>	<i>Pseudozyma hubeiensis</i>	<i>Dothideomycetes sp</i>
<i>Gymnoascaceae sp</i>		<i>Sympoventuriaceae sp</i>	<i>Curvularia pseudorobusta</i>	<i>Claviceps pusilla</i>
<i>Aspergillus tamarii</i>		<i>Aureobasidium namibiae</i>	<i>Penicillium multicolor</i>	
<i>Dothideomycetes sp.; Didymellaceae sp. ZLY-2010</i>		<i>Ochroconis musae</i>	<i>Pseudozyma sp</i>	
<i>Aspergillus salwaensis</i>				

**Table 17. Potential MIC-causing Fungi Across all PACAF Locations**

<b>Prevalent MIC-causing Fungi across three PACAF Locations</b>	<b>Mechanism Influencing MIC</b>	<b>Substrates Affected by MIC</b>
<i>Exophiala</i> <sub>11</sub>	Degrades wood and organic materials; forms biofilms	Water and Metal
<i>Dothideomycetes</i>	Often found as pathogens, endophytes, or epiphytes of living plants; also degrades cellulose and other complex carbohydrates. Several species are lichens, while others occur as parasites.	Unknown
<i>Aureobasidium</i> <sub>6</sub>	Forms biofilms by extracellular polymeric substances (EPS)	Aluminum alloy
<i>Toxicocladosporium/Cladosporium</i> <sub>7</sub>	Metal-ion binding via fungal mycelia	Carbon steel and aluminum alloy
<i>Aspergillus</i> <sub>8</sub>	Oxalic acid metabolite	Magnesium alloy
<i>Penicillium</i> <sub>8</sub>	Forms biofilms by EPS	Aluminum alloy
<i>Nigrospora</i>	Found on decayed wood	Unknown

## 4.5 Analysis of Literature for Available UV Inhibiting Additives and Biocides

### 4.5.1 List of UV Inhibiting Additives to Improve Weathering Performance

The results obtained from weathering studies (section 4.1 and 4.2) were discussed with the manufacturer and it is mutually agreed to add UV stabilizer to improve the weathering of the current LTPC composition. Based on the literature search and performance requirements (thermally stable at LTPC process conditions, no adverse interaction with the ingredients present in the LTPC composition), Battelle down selected potential UV inhibition additives and presented in Table 18.

Table 18: Typical UV absorbers and HALS used in powder coatings<sup>11</sup>

Class	Chemical Name	CAS	Melting range (°C)
Hindered amine class (HALS)	Bis (1, 2, 2, 6, 6-pentamethyl-4-piperidinyl)-[[3, 5-bis (1, 1-dimethylethyl)-4-hydroxyphenyl]methyl]butylmalonate	63843-89-0	146 - 150
	Polymer of 2,2,4,4-tetramethyl-7-oxa-3,20-diaza-dispiro [5.1.11.2]-heneico-san-21-on and epichlorohydrin	202483-55-4	>148°C (softening point)
	Tetramethyl-7-oxa3,20-diazadispro[5.1.11.2]-henicosan-21-one	64338-16-5	>225°C
UV Absorber	2-Ethyl,2'ethoxy-oxalanilide	23949-66-8	126-128°C
	2-(2H-benzotriazol-2-yl)-4, 6-bis (1-methyl-1-phenylethyl)phenol	70321-86-7	137 – 141°C

The potential UV inhibition additives will be provided to LTPC manufacturer and assist them to select the top additive for the formulation.

### 4.5.2 List of Microbicides to Improve MIC Performance

Microbicides are increasingly regulated due to their adverse effects on health and environment. Therefore, formulators should be aware of the safety and environmental concerns for specified microbicides used in the formulation.

Table 13 and Table 17 summarize bacteria and fungi, respectively, potentially causing MIC on the AGE. It is apparent wide varieties of organisms are present on the AGE and are possibly the cause for the corrosion. Therefore, it is critical to control these microbes' formation on the coating substrates as these

<sup>11</sup> George Wpych, e. (2015). Handbook of UV Degradation and Stabilization 2nd edition. Toronto: Chemtech publishing.

microbes are potentially contributing to the corrosion of the coatings. Battelle identified a preliminary list of microbicides (Table 19) that are typically added to coatings to reduce or eliminate microbial growth.

**Table 19. Microbicides used in coating formulations**

Name	CAS	Melting Point	Boiling Point	Vapor Pressure	Chemistry, Mode of Action
3-Iodo-2-propynylbutylcarbamate (IPBC)	55406-53-6	~65-67°C		2.4-4.5x10 <sup>-3</sup> (25°C)	<b>Carbamate; Mode of Action:</b> Disturbs the cell membrane permeability and affects the fatty acids metabolism
Thiabendazole (TBZ)	148-79-8	~297-298°C		4.6-5.3x10 <sup>-7</sup> (25°C)	<b>Benzimidazole; Mode of Action:</b> Contact and systemic fungicide; interferes with the assembly of microtubules (components of fungal exoskeleton)
Carbendazim (BCM)	10605-21-7	~302-307°C		1.5x10 <sup>-4</sup> (25°C)	<b>Benzimidazole, Carbamate; Mode of Action:</b> Interferes with DNA synthesis, mitosis, cytokinesis or related processes
Zinc Pyrithione (ZnP)	13463-41-7	≥240°C		1x10 <sup>-6</sup> (25°C)	<b>Pyridine-N-oxide; Mode of Action:</b> Membrane-active substance with chelating properties; influence on ATP levels, protein synthesis and nutrient transport
2-n-octyl-4-isothiazolin-3-one (OIT)	26530-20-1		120°C	4.9x10 <sup>-3</sup> (25°C)	<b>Isothiazolinone; Mode of Action:</b> Electrophilic active agent. Reacts with nucleophiles (e.g. amines or thiols); enzyme
4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT)	64359-81-5	~41-42°C		9.8x10 <sup>-2</sup> (25°C)	<b>Isothiazolinone; Mode of Action:</b> Electrophilic active agent with activated N-S bond and vinyl activated halogens; reacts with nucleophilic elements of cell proteins resulting in interruption of

Name	CAS	Melting Point	Boiling Point	Vapor Pressure	Chemistry, Mode of Action
					important metabolic processes
2-Butyl-1,2-benzisothiazolin-3-one (BBIT)	4299-07-4		Decom. before boiling	$1.5 \times 10^{-2}$ (25°C)	<b>Isothiazolinone; Mode of Action:</b> Electrophilic active agent
Tebuonazole	107534-96-3	105°C		$1.7 \times 10^{-6}$ (20°C)	<b>Azole; Mode of Action:</b> Inhibition of ergosterol biosynthesis in fungal membranes; at lower azole concentration, retardation of fungal growth, at higher azole conc. Disruption of fungal membranes
Propiconazole	60207-90-1		>250°C	$5.6 \times 10^{-5}$ (25°C)	<b>Azole; Mode of Action:</b> Inhibition of ergosterol biosynthesis in fungal membranes; at lower azole concentration, retardation of fungal growth, at higher azole conc. Disruption of fungal membranes
Silver nanoparticles	Multiple CAS# based on the chemistry		Not Applicable	Not Applicable	<b>Multiple mode of action</b> and well documented additive for inhibiting bacterial, algal and fungal growth
Quat Salts	Multiple CAS# based on the chemistry		Not Applicable	Not Applicable	Disturbs the cell membrane permeability and affects the fatty acids

Considering the broad spectrum of microorganisms present in the environment, a combination of microbial additives is necessary to improve the MIC performance. Furthermore, the additives should be compatible with the LTPC process condition and should not have any adverse environmental and regulatory aspects of the microbicides. Based on the above discussion, we suggest inclusion of silver nanoparticles, Quat Salts, and IPBC in the LTPC formulation.

#### 4.5.3 Applicable Environmental Regulations to Additives and Biocides

It is important to consider the addition of a biocide to provide a long-lasting coating must be balanced with their health effects and potential damage to the environment. There has been a history of additives used in years past like mercury salts, Carbendazim (BCM), and chlorothalonil (CTL) that have been linked to

toxicity, genetic defects, and carcinogenicity. (Shauer, 2017) A few of the microbicides listed are skin sensitizers and care must be taken especially during the coating application whereupon it is airborne. In regard to general structure toxicity, these particular chemistries were not listed by CAS number in California's Proposition 65 (updated December 2017). As with the chemistry, and with specific state regulations, their individual toxicological review may be incomplete and may not be fully representative of actual effects on humans. The Globally Harmonized System of hazard communication details environmental and specific health hazards. It has been adopted to some degree in all major countries, including United States, Japan, and Korea. It may be projected as to what concentration would trigger the requirement of a health hazard pictogram.

Name	CAS	GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
<b>IPBC</b>	55406-53-6	Acute toxicity, Oral (Category 4), H302 Serious eye damage (Category 1), H318 Skin sensitisation (Sub-category 1B), H317 Specific target organ toxicity - repeated exposure (Category 1), H372 Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410
<b>TBZ</b>	148-79-8	Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410
<b>BCM</b>	10605-21-7	Germ cell mutagenicity (Category 1B), H340 <b>Reproductive toxicity (Category 1B), H360</b> Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410
<b>ZnP</b>	13463-41-7	Acute toxicity, Oral (Category 3), H301 Acute toxicity, Inhalation (Category 3), H331 Serious eye damage (Category 1), H318 Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410
<b>OIT</b>	26530-20-1	Acute toxicity, Oral (Category 4), H302 Acute toxicity, Inhalation (Category 3), H331 Acute toxicity, Dermal (Category 3), H311 Skin corrosion (Category 1B), H314 Serious eye damage (Category 1), H318 Skin sensitisation (Category 1), H317 Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410
<b>DCOIT</b>	64359-81-5	Acute toxicity, oral(Category 4), H302 Skin corrosion/irritation(Category 2)H315 Serious eye damage/eye irritation(Category 2A ) H319 Acute toxicity, inhalation(Category 4)H332 Specific target organ toxicity, single exposure(Category 3)H335
<b>BBIT</b>	4299-07-4	Acute toxicity, oral(Category 4), H302 Skin corrosion/irritation(Category 2), H315 Serious eye damage/eye irritation(Category 2A), H319 Specific target organ toxicity, single exposure(Category 3), H335
<b>Tebuonazole</b>	107534-96-3	Acute toxicity, Oral (Category 4), H302 Reproductive toxicity (Category 2), H361 Acute aquatic toxicity (Category 2), H401 Chronic aquatic toxicity (Category 2), H411
<b>Propiconazole</b>	60207-90-1	Acute toxicity, Oral (Category 4), H302 Acute toxicity, Inhalation (Category 2), H330

Name	CAS	GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
		Skin sensitisation (Category 1), H317 Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410

GHS labeling pulled from safety data sheets from concentrated ingredients.

## 4.6 LTPC Manufacturer's Statement on Compatibility of Additives

This task required that the Contractor provide a written and signed statement from the manufacturer of the LTC indicating that the additive and inhibitors presented in this report to AFLCMC/WNZE and those in considerations for modification of formulations for the subsequent laboratory testing tasks are compatible with the LTPC system, and appropriate for PACAF AGE environmental applications. Accordingly, Battelle has received a letter from Hentzen Coatings and it is included in Appendix A.

## 5.0 Conclusions

In terms of UV degradation of the coatings we see a significant loss of gloss across AGE inspected at all the three PACAF locations. We measured the AGE in this region falling below the minimum gloss specification of 15 units for semi-gloss in the 60° angle of incidence as quickly as 3 months on AGE in the PACAF region. This is a sign of UV degradation as the chain scission takes place in the backbone of the polyurethane the bonding between the pigment and resin is reduced. This can cause a chalking and roughening of the coating surface which reduces the gloss of the coating.

We also notice a change in the color of the coating as it sets in the harsh environmental conditions of this region. We see a lightening of the topcoat color as well as the color moving toward the red and yellow color spectrum. This is a sign of UV degradation as polyurethanes will tend to amber with exposure to UV radiation.

Several modes of corrosion were observed in the inspection of the AGE in the PACAF region. It is difficult to determine the exact cause of the corrosion product and impossible to associate it to MIC during visible inspection. Most of the corrosion emanating from fasteners and metallic joints is likely crevice or galvanic corrosion or a combination of the two modes. It appeared there is preferential weld corrosion in couple of cases as the metal used to make the weld is slightly anodic compared to the parent metal. Therefore, the weld metal corrodes at a higher rate than the parent. Corrosion appearing through paint at corners and edges is likely a result of edge effects and the coating naturally being thinner there, so it is the first spot to form pores and thus lead to corrosion propagation along the edge (and out). The corrosion where the paint is bubbling up is likely filiform corrosion but could be MIC.

As for the potential organisms that may contribute to the corrosion, there have been numerous studies demonstrating that microorganisms may be involved in corrosion. For example, bacteria belonging to the genus *Sphingomonas* were detected in 2011 in an underground pyrite mine.<sup>12</sup> The bacteria were detected in acid streamer samples at pH 3, and resulted in better growth under microaerobic and anaerobic conditions than when grown aerobically. Colonies of the sample were gelatinous, and liquid cultures were highly viscous, suggesting the sample produces exopolymeric materials, which is a common characteristic of *Sphingomonas* spp. These characteristics are all indicative of a type of bacteria that can produce a type of biofilm, grow anaerobically (i.e. without oxygen), and live in a highly acidic environment. These are all essential characteristics of an organism that helps to promote MIC. As was shown in Table 10-Table 12, bacteria from the *Sphingomonas* genus were found most frequently across

<sup>12</sup> Kimura *et al.*, Biodiversity and geochemistry of an extremely acidic, low-temperature subterranean environment sustained by chemolithotrophy. 2011. Environmental Microbiology. 13(8), 2092–2104.

all three sites and across all AGE samples. Ultimately, as listed in Table 13, *Sphingomonas*, along with four other organisms, was selected for testing to observe whether it contributes to corrosion. *Acidovorax* is known to be an IOB as well as a nitrate reducer, and *Variovax* is known to produce biofilms and degrade copper. The bacteria that belong to the genus *Pseudomonas* are another strong candidate to contribute to MIC, as they are known to form biofilms and contribute to stainless steel, carbon steel, and copper corrosion. They can also reduce nitrate. All of these characteristics make it very likely to be a contributor to MIC. Finally, bacteria belonging to *Massilia* and *Methyllobacterium* genuses were selected as candidates. They are found in MIC-causing consortia, and are both known to degrade copper.

Next, the fungi that potentially contribute to corrosion were considered. They are listed in Table 14-Table 16, and the downselected group is listed in Table 17. All fungi found in these genuses are found across the sites as well as across the AGE samples. These fungi are known to form biofilms and/or degrade wood or other organic materials. These candidates are the most promising in terms of potentially contributing to corrosion.

## 6.0 References

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## Appendix A: Hentzen Coatings Letter per the AF PWS requirements



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January 12, 2018

**SUBJECT:** Additive Statement per PWS 4.3.1 for Phase II PR Number: FD2060-17-30718

Dear Vinay,

Hentzen Coatings, Inc. has researched and down-selected commercially available UV and MIC additives compatible with Commercial Off The Shelf Low Temperature Powder Coating (COTS LTPC) formulations.

A Hindered Amine Light Stabilizer (HALS) will be used to improve weatherability from UV degradation.

3 down selected additives for inhibiting Microbial Induced Corrosion (MIC) will be approved by Battelle microbiologists for testing in the following test systems in Air Force Color 26173 Semi-Gloss Gray:

- 1) Control - COTS LTPC (No UV or MIC Additives)
- 2) COTS LTPC + HALS
- 3) COTS LTPC + MIC Inhibitor #1 + HALS
- 4) COTS LTPC + MIC Inhibitor #2 + HALS
- 5) COTS LTPC + MIC Inhibitor #3 + HALS
- 6) COTS LTPC (Anti-Microbial resin modification to reduce MIC)

If you have any additional questions, call me or Andy Daly at (414) 353-4232 [adaly@hentzen.com](mailto:adaly@hentzen.com).

Best Regards,

A handwritten signature in black ink that reads "R.J. Pomykala".

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ISO 9001:2008  
AS9100 REV.C

## Appendix B: Coatings thickness measurements recorded on the inspected AGE at the three PACAF locations

Location	Equipment		Coating Thickness Measurement (mils)						
	Nomenclature	Serial #	Sampling Location	#1	#2	#3	AVG		
Kadena South	C10 aircart	AU 21	side	7.2	8		7.6		
			axle	6.1	6.4	7.2	6.6		
Kadena South	Generator	LB 25	bumper	4.3			4.3		
			tow bar	6	4.8		5.4		
			axle	9.4	5.1	6	6.8		
Kadena South	Generator	GT 28	tow bar	4.2	3.4	2.3	3.3		
			frame	2.7	2.4	2.5	2.5		
			fuel tank strap	7.6	8.1	3.1	6.3		
Kadena North	B1 Stand	B1 64	Frame around standing area						
				5.1	3.9	5.9	5.0		
Kadena North	AC + Heat Veh	AA 05	axle	17.9	9	13.8	13.6		
			Back Bumper	3.8	7.6	6.8	6.1		
Kunsan	Dash 60	TG 27	frame	25	25.8	21.1	24.0		
			Left side frame	5.8	4.8	10.2	6.9		
			front tow bar	15	12.3	12.8	13.4		
			tank strap	5.3	2.3	2.4	3.3		
Kunsan	Dash 95	TC - 21	tow bar	2.9	3.6	4.7	3.7		
			right side frame	9.9	9	7	8.6		
			front	6.5	8	6.1	6.9		
Kunsan	7000# bomb lift	MH-10	Back side above exhaust						
				7	5.6	6.3	6.3		
			top	3	3.8	7.6	4.8		
Kunsan	C1 stand	C1-12	top frame	6.4	6.7	4.3	5.8		
			left side	5	2.2	1.5	2.9		
			right side	1.9	1.1	1.6	1.5		
Kunsan	Load Bank	LB-04	front step	1.2	1.1	2.5	1.6		
			Front frame low						
				1.7	1.7	1.4	1.6		
Kunsan			back colling grate						
				1.9	2.7	2.4	2.3		
			top	2.2	3	2.6	2.6		

Location	Equipment		Coating Thickness Measurement (mils)				
	Nomenclature	Serial #	Sampling Location	#1	#2	#3	AVG
Andersen	MU-1C bomblift	MUW6	top	15.2	11.7	9.3	12.1
			right side	14.4	16.4	21.7	17.5
			left side	9.6	6.6	8.2	8.1
Andersen	Dash 86	PC-03	tow bar	5.5	6	6.4	6.0
			left side	8.2	9.6	9.9	9.2
			right side	7.2	8	8	7.7
Andersen	809 Generator	B8W4	left side	3.5	4.9	4.9	4.4
			top	5.3	5.9	4.3	5.2
			right side	5.3	4.6	5.1	5.0
Andersen	Dash 86	PC-70	tow bar	4.1	3.9	4.2	4.1
			top	8.5	8.5	11	9.3
			back side	6.2	6.2	6.5	6.3
Andersen	85 stand	B5-97	left side	4.2	4.3	3.1	3.9
			right side	3.3	3.6	3.5	3.5
			front side	3.5	5.4	4.6	4.5